PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : A01H 5/00, C12N 15/29, 15/31, 15/54, 15/70, 15/74, 15/80, 15/81, 15/82, C12P 19/04

A1

(11) International Publication Number: WO 98/44780

(43) International Publication Date: 15 October 1998 (15.10.98)

(21) International Application Number: PCT/US98/06660

(22) International Filing Date: 3 April 1998 (03.04.98)

(30) Priority Data:
60/042,939 4 April 1997 (04.04.97) US

(71) Applicant *(for all designated States except US)*: EXSEED GENETICS, LLC [US/US]; Iowa State University, 1575 Food Science Building, Ames, IA 50011-1061 (US).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: GUAN, Hanping [CN/US]; 1608 Crestwood Circle, Ames, IA 50010 (US). KEELING, Peter, L. [GB/US]; 3409 Oakland Street, Ames, IA 50011 (US).

(74) Agent: SADOFF, B., J.; Nixon & Vanderhye, P.C., 8th floor, 1100 North Glebe Road, Arlington, VA 22201 (US).

(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, GW, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: PLANT LIKE STARCHES AND THE METHOD OF MAKING THEM IN HOSTS

(57) Abstract

This invention relates to hosts containing constructs with genes from the starch pathway. More typically the present invention relates to bacterial hosts that form plant like starches. Additionally the present invention relates to plant hosts that have genes from the starch pathway. The invention further relates to the starches produced by said hosts.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
AM	Armenia	FI	Finland	LT	Lithuania	SK	Slovakia
AT	Austria	FR	France	LU	Luxembourg	SN	Senegal
AU	Australia	GA	Gabon	LV	Latvia	SZ	Swaziland
AZ	Azerbaijan	GB	United Kingdom	MC	Monaco	TD	Chad
BA	Bosnia and Herzegovina	GE	Georgia	MD	Republic of Moldova	TG	Togo
BB	Barbados	GH	Ghana	MG	Madagascar	TJ	Tajikistan
BE	Belgium	GN	Guinea	MK	The former Yugoslav Republic of Macedonia	TM	Turkmenistan
BF	Burkina Faso	GR	Greece			TR	Turkey
BG	Bulgaria	HU	Hungary	ML	Mali	TT	Trinidad and Tobago
BJ	Benin	IE	Ireland	MN	Mongolia	UA	Ukraine
BR	Brazil	IL	Israel	MR	Mauritania	UG	Uganda
BY	Belarus	IS	Iceland	MW	Malawi	US	United States of America
CA	Canada	IT	Italy	MX	Mexico	UZ	Uzbekistan
CF	Central African Republic	JP	Japan	NE	Niger	VN	Viet Nam
CG	Congo	KE	Kenya	NL	Netherlands	YU	Yugoslavia
CH	Switzerland	KG	Kyrgyzstan	NO	Norway	ZW	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's Republic of Korea	NZ	New Zealand		
CM	Cameroon			PL	Poland		
CN	China	KR	Republic of Korea	PT	Portugal		
CU	Cuba	KZ	Kazakstan	RO	Romania		
CZ	Czech Republic	LC	Saint Lucia	RU	Russian Federation		
DE	Germany	LI	Liechtenstein	SD	Sudan		
DK	Denmark	LK	Sri Lanka	SE	Sweden		
EE	Estonia	LR	Liberia	SG	Singapore		

PLANT LIKE STARCHES AND THE METHOD OF MAKING THEM IN HOSTS

The present application is based on U.S. Provisional Application No. 60/062,939, filed April 4, 1997, the entire contents of which is incorporated hereby by reference.

BACKGROUND -- FIELD OF INVENTION

This invention relates to hosts containing constructs with genes from the starch pathway. More specifically the present invention relates to bacterial hosts that form plant like starches. Additionally the present invention relates to plant hosts that have genes from the starch pathway. The invention further relates to the starches produced by said hosts.

BACKGROUND -- DESCRIPTION OF PRIOR ART

The starch using industry includes diverse industries such as candy makers, makers of adhesives and paints, gravy makers, paper producers, etc. Since the demand for starch, (which is formed of amylose and amylopectin), has been dramatically increasing for specialized food and industrial uses, efforts have been undertaken to tailor the quantity and quality of starch for specific food and industrial uses.

This industry has overtime looked for a number of different starches having, high viscosity, lower viscosity, higher gelling strength and lower gelling strength, different boiling points etc. Each starch tailored for a number of uses. The industry has utilized mutant starches that have less amylopectin and mutant starches with more amylose for tailored specifications. For example the increased amylose starch has been used in the gelled candy making area. And the industry has used the increased amylopectin starches formed by mutants such as wx and wx su2 containing little amylose and mostly amylopectin for thicken foods like pudding, pies, gravies, frozen foods and batters, stews, canned foods and baby food. Additionally the mutant starches of different types have usefulness as adhesives and as sizing.

The other method used to address the industry needs for tailored starch is the use of chemical modification of the starch. Chemical derivation of the starch are produced by chemically reacting the starch with the monofunctional reagents to introduce the substituents

such as phosphate, acetate, succinate groups to stabilize the starch. Unfortunately, these types of starches can be subjected to government regulation and additionally have less acceptance generally due to the added cost of the starch.

Starch is the major form in which carbohydrates are stored in biological systems. Plant starch in chloroplasts is transitory and storage starch accumulates in storage organs of many plant. Starch can be found in all organs of most higher plants including leaves stems and roots and fruits and embryo and endosperm. In addition to higher plants starch, similar polysaccharide (glycogen) has been found in bacteria. Many bacteria produce a reserve polysaccharide similar to the glycogen found in animals.

Storage polysaccharide has been classified as being in two groups, group one has storage in the cytsol of the cell and the second group within the plastid. Escherichia coli produces a polysaccharide within the cytsol. Starch producing plants typically store starch in the plastids. Typical starch bearing plants include cassava, potato, corn, peas, rice, wheat, barley. The main starch storing tissue of corn, rice wheat and barley and oats, the cereal grains, is the endosperm.

Starches are also classified by the plant source, for example cereal starches are from cereal grains such as maize, rice, wheat, barley, oats and sorghum; tuber and root starch are from potatoes and yams and cassava .

The pathway of starch synthesis is not well understood. Generally, as noted above starch from plants, consists of two major components: amylose and amylopectin. These intertwine in the starch granule of the plants. Amylose is a linear polymer of alpha 1 -4 bonded anhydroglucose units while amylopectin is a branched polymer comprised of linear chains alpha 1-4 linked anhydroglucose units with branches resulting from alpha1-6 linkages between the linear chains. It has been known for sometime that mutant genes in starch bearing plants can be expressed and that the properties of the starch can be altered. The proportion of the two components and their structures in the mutant primarily determine the physical-chemical properties of the starch.

Thus the lack of a clear understanding of the starch synthesis pathway and the difficulty of employing mutants limited the industry to the use of existing and producible mutant starches (cereals containing mutant starch can show a tremendous yield penalty in field environments) or to the chemical modification that could be made to the starch. In the last decade the industry has been studying the effects of certain starch genes in plants and bacteria in an attempt to more clearly understand starch synthesis.. Since the late 80's it has been possible to transform plants and bacteria to contain isolated genes. In response to this the industry has transformed potatoes with a bacterial gene GS and with starch soluble synthase III gene in the antisense (forming a mutant). As part of these potato starch experiments bacteria has been transformed with certain potato starch genes. For example the SSSIII gene from potato was transformed into E.coli deficient in glgA gene. The effect of glgC and branching enzyme I and II in combination in a mutant E.coli has also been studied and glycogen like product was reported. The starch industry that is commercial does not have a particular interest in the production of glycogen which is the polysaccharide produced by bacteria and animals (the health care industry may have some such interest). The industry has thus not yet been able to generate tailored starches at reasonable prices through plant gene transformation. There remains a need for the industry to find new starches that are useful due to their changed characteristics such as lower viscosity and new starches that are useful because of their higher viscosity and new methods of producing such starches.

Glycogen synthesis in *E. coli* and starch synthesis in higher plants have similar pathways involving ADPGlc pyrophosphorylase, starch synthase,(SS) or glycogen synthase (GS,) and branching enzyme (BE). It has been suggested that ADPGlc pyrophosphorylase plays a pivotal role in regulating the amount of starch synthesized, while starch synthase and starch branching enzyme primarily determine the starch structure. Multiple forms of SBE and SS have been identified in many plants including maize, rice, pea and potato. In addition to the waxy gene coding for granule bound starch synthase (GBSS), three genes coding for the other forms of SS have been isolated from maize endosperm. Maize is the only cereal crop from which the genes coding for the five forms of SS have been isolated. Clearly a number of these sequences are published and known to those of skill in the art. Genes coding for maize SBE have also been cloned and characterized. Previous reports have demonstrated that maize SBEI

has a higher rate of branching amylose than SBEII and preferentially transfers longer chains, while SBEII shows a higher rate of branching amylopectin and preferentially transfers shorter chains. In comparison with SBE, less is known about the specificities and functions of multiple forms of SS. In *Waxy* maize, which lacks GBSS, only amylopectin is synthesized and amylose is missing. Therefore, it is generally accepted that GBSS, encoded by *waxy* gene, is primarily responsible for the synthesis of amylose. Study of *waxy* mutation in *Chlamedomonas reinhardtii* has suggested that GBSS is also involved in amylopectin synthesis. Although it has been reported that *Chlamedomonas reinhardtii* SSII controls the synthesis of intermediate size glucans of amylopectin in *Chlamedomonas*, direct evidence for the functions of SS in higher plants is still missing. Antisense technology has been used to study the functions of SS in potato, however, the results are inconclusive.

In an article written by Hanping Guan et al., entitled AMaize Branching Enzyme Catalyzes Synthesis of Glycogen-like Polysaccharide in *glg B*-deficient *Escherichia coli@*. Published in Proc. Natl. Acad. Sci. USA, Vol. 92, pp. 964-967, February 1995 Plant Biology a specific glycogen like polysaccharide from a transformed E coli was reported. This article taught the transformation of an E coli bacteria with maize BEI and BEII. These enzymes were transformed into two E coli hosts. One of the bacterial hosts was a wild type and the other was a mutant. The mutation to the bacteria was the reduction of the activity of glycogen BE in the AC71(glgB-) so that the mutant was essentially free of BE activity. The paper analyzed the debranched alpha-glucan isolated from the four different transformants. The first host was E. coli containing glgB and the second host was the AC71 without any transformed genes then AC71 transformed with maize BEII, and then AC71 with maize BEI , then AC71 with maize BEI and BEII. The resultant polysaccharide products were analyzed by HPLC, by chain length and relative peak area and by mole distribution of chains. The study led to the understanding that BEII could play a role in synthesis of the short chains of amylopectin and BEI could be involved with the longer chains of amylopectin. The paper also noted that the mutant host AC71 produced more chains with chain length of 6 then did the wild type E. Coli. The paper also noted that the maize BE and the GS of the host **did not** produce amylopectin like polysaccharides. The article suggested that the concerted action of GS with different BE=s could play an important role in determining the final structure of the polysaccharide synthesized. The article by Guan ends by suggesting that his study had established the basis for

studying the concerted actions of BE and SS in a bacterial model system.

The expression of E coli GS (glycogen synthases) in potatoes showed a large incidence of highly branched starch. This result was published in an article in Plant Physiol. 104,1159-1166 by Shewaker et al. This potato does not appear to be of much commercial use at this time.

The industry still needs the option of producing plant like starches in a fermentation process from bacteria and thus without the necessity of breeding and growing environment sensitive plants; and, the option of producing plants that generate the specific tailored starch through a plant host. And the industry needs altered and new starches that are cereal like starches or root and tuber like starches in large quantities and inexpensively thus avoiding having to use chemical modification of starch. The industry needs a host that can be readily transformed to supply different starches tailored to the industry=s need. Specifically the industry needs a host that supplies various different starches including those not capable of being made in plants or bacteria presently.

OBJECTS AND ADVANTAGES

Accordingly, several objects and advantages of the invention are to produce plant like starch through the process of fermentation.

Additional objects and advantages are the production of new starches in plants.

Still further objects and advantages will become apparent from a consideration of the ensuing description and accompanying drawings.

Another object of the present invention is the synthesis of polysaccharides including amylose, amylopectin in E. coli, and/or fungal and yeast by plant starch synthesizing enzymes including SS, SBE, bacterial branching enzyme, glycogen synthase and other enzymes in other living organism.

Yet another object of my invention is using each or combination of these enzymes ***or***

modified enzymes studied in this patent to produce or to improve polysaccharides in any living organism including starch synthesis in plants.

SUMMARY OF THE INVENTION

The invention provides DNA constructs in a host that include most of the genes in the starch pathway of a plant such that the host produces a plant like polysaccharide. And in one embodiment produces maize starch including slightly different embodiments that make specific maize mutant like starch in a non plant host. This invention encompasses a bacterial host containing a combination of two or more of such genes SSI, SSSIIa, SSIIb, SSSIII, GBSS, BEI and BEII when the combination does not form glycogen like material. This invention encompasses a plant host transformed with any of the following maize genes or a plant host having a combination of two or more of the following maize genes SSI, SSIIa, SSIIb, SSSIII, GBSS, BEI and BEII in a hybrid or an inbred rice plant.

Additionally the present invention includes new`polysaccharide produced by a transformed host. The host having a wildtype, which does not produce the new polysaccharide, the transformed host expressing at least two exogenous starch synthesis genes, the genes are selected from a group consisting of starch synthesis genes such as SSI SSIIa, SSIIb, SSIII, GBSS and optionally including at least one of the BEI and BEII genes wherein the transformed host is capable of producing such new polysaccharide.

The invention also covers a new polysaccharide wherein the host also expresses the exogenous genes selected from the following group consisting of bacterial glycogen inducing genes are selected from the group glgA, glgB, glgC and any mutants thereof. Or wherein the host also expresses the exogenous genes selected from the following group consisting of plant granule bound enzymes. And the new polysaccharide wherein the starch synthesis genes are selected from the group consisting of BEI and BEII.

The present invention broadly encompasses a host containing a transformed Glg C gene and at least one of the starch branching enzymes genes in a host in combination with at least one other transformed starch gene wherein the host produces a polysaccharide product. And a host containing transformed bacterial gene and at least one of the non starch branching

enzymes selected from the group consisting of debranching enzymes and soluble starch synthase

A method of producing polysaccharides which are non glycogen like in a host comprising transforming a host capable of being used in a fermentation process, with genes selected from the group which produce starch synthesizing enzymes, or glycogen synthesizing enzymes such that the host produces nonglycogen like starch, and employing the host in a fermentation process that produces polysaccharides. The host is bacteria, or a fungal or a yeast. Additionally the method of this invention includes the use of bacterial genes also such as the glycogen synthesizing genes including the glgC, glgA, glgB genes. A method wherein the genes which produce starch synthesizing enzymes include genes encoding for starch soluble synthases I, IIa, IIb and SS III (dul1). A method wherein the genes which produce starch synthesizing enzymes include genes encoding for starch debranching enzyme and branching enzymes. ***The invention covers the modified starch synthesizing enzymes including the N-terminally truncated SS.***

In other word the invention covers a host transformed to carry a gene active in glycogen production, and at least one nonstarch branching gene active in the production of at least one of the following polysaccharides amylopectin and amylose in its original host. The host can be a monocot or a dicot plant. The host can be a cereal bearing plant. Or the host can be a bacteria.

More specifically the invention includes a host wherein at least one nonstarch genes active in the production of at least one of the following polysaccharides, amylopectin and amylose in its original plant, is selected from the group consisting of starch soluble starch synthase I, Iia, IIb, III genes and debranching enzyme gene (su1), GBSS gene, sh2 gene and bt2 gene. A host including at least one of the starch branching enzyme genes such as BEI gene, BEII gene.

The present invention can also be described as a host transformed to carry a gene active in ADPG production, and at least one starch gene active in the production of at least one of the following polysaccharides amylopectin and amylose in its original host wherein the host

produces polysaccharides that are plant like starch and not glycogen like.

Additionally the host can be transformed to carry a pyrophosphorylase gene, and glycogen synthase gene.

The scope of the present invention includes a host deficient in alpha 1,4 glucan synthesizing ability and alpha 1,4-1,6 branching enzyme capability transformed to express at least one a plant starch soluble synthesis gene. And the host can also include being transformed to express at least one gene encoding for debranching enzyme, and/or a gene encoding for starch soluble synthase I, starch soluble synthase enzyme Iia, IIb, starch soluble synthase enzyme III. This host can including being transformed to express at least one gene encoding for starch branching enzyme.

This invention also includes the production of a glycogen like material in plants. Attached hereto are a number of plasmids described by the figures and by table one, that are part of the present invention and are claimed herein. One such example is the plasmid wherein the plasmid is in a carrier host and the plasmid contains the SSIIa gene with the n terminus GENVMNVIVV and wherein the gene is approximately 1561 base pairs in length. The invention includes mutant hosts such as mutant plants like waxy rice and potatoes and corn as example and wherein the host is a mutant E. Coli, or fungus.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 shows a graph which gives the relative peak area in percent and the chain length of glycogen and starch soluble synthase I (SSI), starch soluble synthase II (SSIIa), starch soluble synthase IIb (SSIIb). Thus this shows the specificities of Maize SS=s in chain elongation.

FIG. 2 shows plasmid pEXSC-MBEI with 7661 base pairs and promoter T7 and a Kanamycin gene and glgC and the maize starch branching enzyme I (MBEI).

FIG. 3 shows plasmid pEXSC3C with 7461 base pairs and promoter T7 and ampicillin

gene and the maize starch soluble synthase gene Iia. pEXS3c is the 1082 bp Nde I-EcoRI fragment containing the N-terminus of MSSSIIa (from MSSIIa in pBSK) subcloned into the Nde I-EcoRI sites of pEXS3a, replacing the N-terminus of IIA-2 with the longer IIa N-terminus. MSSIIa is the mature maize SSIIa and is 2090 bp long. The following sites are not contained in the MSSIIc insert: Apa I, BglII, Eco V, Not, Spe I, and Xba I. The N-terminus of this plasmid is AEAEAGGKD.

FIG. 4 shows plasmid pEXSC-MBEI-MBEII with 9971 base pairs and promoter T7 and a Kanamycin gene and glgC and the maize starch branching enzyme I (MBEI) and the maize starch branching enzyme II (MBEII).

FIG. 5 shows plasmid pEXSC-MBEII with 7521 base pairs and promoter T7 and a Kanamycin gene and glgC and the maize starch branching enzyme II (MBEII).

FIG. 6 shows plasmid pEXSC-3a with 7990 base pairs and promoter T7 and a Kanamycin gene and the glgC gene and the maize N-terminally truncated starch synthase gene IIa (MSSIIa-2). The N-terminal sequence is GENVMNVI.

FIG. 7 shows plasmid pEXSC-8 with 7079 base pairs and promoter T7 and a Kanamycin gene and the glgC gene and the maize starch soluble synthase gene I and version I-2.(MSSI-2), An N-terminally truncated SSI.

FIG. 8 shows plasmid pEXSC-9 with 7551 base pairs and promoter T7 and a Kanamycin gene and the glgC gene and the maize starch soluble synthase gene IIb (SSIIb). The N-terminal sequence is AAAPAGEE.

FIG. 9 shows plasmid pEXSC-10 with 7211 base pairs and promoter T7 and a Kanamycin gene and the glgC gene and the maize starch soluble synthase gene I, the full length SSI. The N-terminal sequence is CVAELSREGPA

FIG. 10 shows plasmid pEXSCA with 6738 base pairs and promoter T7 and a Kanamycin gene and the glgC gene and the glgA gene.

FIG. 11 shows plasmid pEXSC9a with 7240 base pairs and promoter T7 and ampicillin gene and the maize starch soluble synthase gene IIb-2 (Maize SS IIb-2), an N-terminally-truncated SSIIb. The N-terminal sequence is MNVVVVASECAP.

FIG. 12 shows plasmid pEXSWX with 6968 base pairs and promoter T7 and an ampicillin gene and the N-terminally-truncated maize WX (maize granular bound starch synthase). The N-terminal sequence for wx is ASAGMNVVFVGAEMA.

FIG. 13 shows plasmid pEXSWX2 with 6980 base pairs and promoter T7 and an ampicillin gene and the N-terminally-truncated maize WX termed as wx2. The N-terminus of wx2 is MNVVFVGAEMA.

FIG. 14 shows plasmid pEXSC9 with 7780 base pairs and promoter T7 and ampicillin gene and *E. coli glgC* gene and the maize starch soluble synthase geneIIb (Maize SS IIb).

FIG. 15 shows plasmid pEXSC10d with 7112 base pairs and promoter T7 and ampicillin gene, *E. coli glgC* gene and the N-terminally-truncated maize starch soluble synthase gene I termed as Maize SSI-3). The N-terminus of maize SSI-3 is MSIVFTGEASPYA.

FIG. 16 shows plasmid pEXS10 with 5300 base pairs and promoter T7 and ampicillin gene and the full length maize starch soluble synthase gene I termed as Maize SS I.

FIG. 17 shows plasmid pEXS8 with 7259 base pairs and promoter T7 and ampicillin gene and the N-terminally-truncated maize starch synthase gene I termed as SSI-2. The N-terminal sequence is CVAELSRDLGLEPEG.

FIG. 18 shows plasmid pEXSCA1 with 5128 base pairs and promoter T7 and ampicillin gene and the glgA. pESCA1 is a 1551 bp SpeI-Sac I fragment containing glgA (from glgA in pBSK) subcloned into the Xba I- Sac I sites of p ET-23d which is commercially available from Novagen in Madision Wisconsin under catalog number 69748-1 and called ET-23d(+) DNA.

FIG. 19 shows the spectrum of the iodine glucan complex of the product produced by the host containing the glgC and glgA, and the pEXSC9, pEXSC3, pEXSC8, pEXSCwx the X-axis is listing nm and the Y axis is reading absorbance.

FIG. 20 shows the spectrum of the iodine glucan complex of the product produced by the host transformed with plasmids containing the glgC, the BEI, the BEII genes and glgA; glgC, the BEI, the BEII genes and maize SSI, SSI-2 and glgC, the BEI, the BEII genes and maize SSIIb, and glgC, the BEI, the BEII genes and maize SSIIa-2, and glgC, the BEI, the BEII genes, the X-axis is listing nm and the Y axis is reading absorbance.

FIG. 21 shows the product produced by the host in small bottles including the product from the host containing glgC, the BEI, the BEII genes and maize SS=s genes. Encoded as (C-I-II+8), glgC, the BEI, the BEII and maize SSI-2 genes and pEXSC10 encoded as (C-I-II+10), glgC, the BEI, the BEII and maize SSI genes and pEXSC9 encoded as (C-I-II+9),glgC, the BEI, the BEII and maize SSIIb genes and pEXSC3a encoded as (C-I-II+3a), glgC, the BEI, the BEII and maize SSIIa-2 genes and pEXSCWX encoded as (C-I-II+WX),glgC, the BEI, the BEII and maize waxy genes and pEXSCA1 encoded as (C-I-II+A1), containing maize BEI, BEII and E.coli glgA genes. potato dextrin, waxy maize starch, corn amylopectin, rice starch, corn starch, pEXSC8.

FIG. 22 shows pExs-trc has 4178 base pairs with the trc promoter and the ampicillin gene. PEXS trc is pTrc99A-Ndel which has been mutagenixed. (Nco I site in multiple cloning site of p Trc99A-Ndel is mutagenixed to Nde I using primers EXS63 AND EXS64.) pEXS-trc contains only one Nde I site and no Nco I sites. The following sites are not contained in pEXS-trc; Bgl II, Cla I, Nco I, Not I, Sac II, SnaB I, Spe I, and Xho I.

FIG. 23 shows pEXS-trc3 has 4129 base pairs with the trc promoter and the ampicillin gene in partial and the Kanamycin gene. The pEXS-trc3 is pEXS-trc1 cut with BglI (filled in)-Sca I and religated, deleting most of the Amp gene (304 nt from the 5" end remain). The following sites are Not contained in p EXS-trc3: Apa I, Bgl II, Eco V, Nco I, Not I, SnaB I, and Spe I.

FIG. 24 shows the plasmid pEXS 102 having 7190 base pairs, adapted for plant transformation containing the maize 10KD zein promoter, and maize adh I intron, the gene coding for the maize starch synthase I transit peptide, and the Waxy 2 gene and the nos terminator and the ampicillin gene.

FIG. 25 shows the plasmid pEXS 103 having 6607 base pairs, adapted for plant use containing the maize 10KD zein promoter,the gene coding for the maize starch synthase I transit peptide and the Waxy 2 gene and the nos terminator and the ampicillin gene.

FIG. 26 shows the plasmid pEXS 101 having 6979 base pairs, adapted for plant use containing the maize 10KD zein promoter, the gene coding for the maize starch synthase I transit peptide and the glg B gene and the nos terminator and the ampicillin gene.

FIG. 27 shows the plasmid pEXS 100 having 7557 base pairs, adapted for plant use containing the maize 10KD zein promoter, and maize adh I intron, the gene coding for the maize starch synthase I transit peptide, and the glg B gene and the nos terminator and the ampicillin gene.

FIG. 28 shows the plasmid pEXS 101 having 6273 base pairs, adapted for plant use containing the maize 10KD zein promoter, the gene coding for the maize starch synthase I transit peptide, and the glg A gene and the nos terminator and the ampicillin gene.

FIG. 29 shows the plasmid pEXS 66 having 6001 base pairs, adapted for plant use containing the maize 10KD zein promoter, the gene coding for the maize starch synthase I transit peptide, and the glg C_3 gene and the nos terminator and the ampicillin gene.

FIG. 30 shows the plasmid pEXS 65 having 6373 base pairs, adapted for plant use containing the maize 10KD zein promoter, the gene coding for the maize starch synthase I transit peptide, and the maize waxy gene and the nos terminator and the ampicillin gene.

FIG. 31 shows the plasmid pEXS 64 having 7073 base pairs, adapted for plant use containing the maize 10KD zein promoter,the gene coding for the maize starch synthase I

transit peptide, and the maize soluble starch synthase IIa gene and the nos terminator and the ampicillin gene.

FIG. 32 shows the plasmid pEXS 63 having 6473 base pairs, adapted for plant use containing the maize 10KD zein promoter, the gene coding for the maize starch synthase I transit peptide, and the maize soluble starch synthase IIa gene and the nos terminator and the ampicillin gene.

FIG. 33 shows the plasmid pEXS 62 having 6773 base pairs, adapted for plant use containing the maize 10KD zein promoter,the gene coding for the maize starch synthase I transit peptide, and the maize soluble starch synthase I-2 gene and the nos terminator and the ampicillin gene

FIG. 34 shows the plasmid pEXS 61 having 7013 base pairs, adapted for plant use containing the maize 10KD zein promoter, the gene coding for the maize starch synthase I transit peptide, and the maize soluble starch synthase IIb gene and the nos terminator and the ampicillin gene.

FIG. 35 shows the plasmid pEXS 59 having 6858 base pairs, adapted for plant use containing the maize 10KD zein promoter, and maize adh I intron, the gene coding for the maize starch synthase I transit peptide, and the E.coli glgA gene and the nos terminator and the ampicillin gene

FIG. 36 shows the plasmid pEXS 58 having 7658 base pairs, adapted for plant use containing the maize 10KD zein promoter, and maize adh I intron, the gene coding for the maize starch synthase I transit peptide, and the maize soluble starch synthase IIa gene and the nos terminator and the ampicillin gene.

FIG. 37 shows the plasmid pEXS 56 having 6586 base pairs, adapted for plant use containing the maize 10KD zein promoter, and maize adh I intron, the gene coding for the maize starch synthase I transit peptide, and the glg C_3 gene and the nos terminator and the ampicillin gene.

FIG. 38 shows the plasmid pEXS 54 having 7658 base pairs, adapted for plant use containing the maize 10KD zein promoter, and maize adh I intron, the gene coding for the maize starch synthase I transit peptide, and the Maize SS IIa gene and the nos terminator and the ampicillin gene.

FIG. 39 shows the plasmid pEXS 53 having 7058 base pairs, adapted for plant use containing the maize 10KD zein promoter, and maize adh I intron, the gene coding for the maize starch synthase I transit peptide, and the maize starch soluble synthase IIa-2 gene and the nos terminator and the ampicillin gene.

FIG. 40 shows the plasmid pEXS 52 having 7358 base pairs, adapted for plant use containing the maize 10KD zein promoter, and maize adh I intron, the gene coding for the maize starch synthase I transit peptide, and maize starch soluble synthase I-2 gene and the nos terminator and the ampicillin gene.

FIG. 41 shows the plasmid pEXS 51 having 7398 base pairs, adapted for plant use containing the maize 10KD zein promoter, and maize adh I intron, the gene coding for the maize starch synthase I transit peptide, and maize starch soluble synthase IIb gene and the nos terminator and the ampicillin gene.

FIG. 42 shows photograph of eleven products of altered starch produced with the present invention. The titled are encoded C-I-II=the glgC gene and the BEI and the BEII genes the following number or alternatively designation means pEXS-and the number. Thus C-I-II=the glgC gene and the BEI and the BEII and EXS-10 plasmid that contains the gene SSI, having the N-terminus shown in Table 1.

FIG. 43 shows the DNA sequence and the protein sequence for glgA having 1488 base pairs.

FIG. 44 shows the DNA sequence and the protein sequence for glgB having 2361 base pairs.

FIG. 45a shows the DNA sequence for Zea mays 10-kDa zein gene having 2562 base pairs .

FIG. 45b shows the DNA sequence for Zea mays 10-kDa zein portion of the gene used as the promoter in a number of the plasmids discussed herein.

FIG. 46 shows the DNA sequence and the protein sequence for glgC3 (glgC_3) having 1328 base pairs containing two mutations P295D, K296E. This is a mutant of the wild type glgC gene.

FIG. 47 shows the DNA sequence and the protein sequence for glgC (glgC) having 1328 base pairs.

FIG. 48 shows the DNA sequence and the protein sequence for glgCwt (glgCwt) having 1328 base pairs. This is the glgC gene that is found in nature.

FIG. 49 shows the DNA sequence and the protein sequence for the maize waxy gene denoted wx herein.

FIG. 50 shows the DNA sequence and the protein sequence for the maize starch soluble synthase IIb encoding gene having 2423 base pairs.

FIG. 51 shows the DNA sequence and the protein sequence for the maize starch soluble synthase IIa.

FIG. 52 shows the DNA sequence and the protein sequence for the maize starch soluble synthase I-2 having 1749 base pairs.

FIG. 53 shows the DNA sequence and the protein sequence for the maize branching enzyme II.

FIG. 54 shows the DNA sequence and the protein sequence for the maize branching enzyme I.

FIG. 55 shows the DNA sequence and the protein sequence (153) for the transit peptide portion of the maize starch soluble synthase I.

FIG. 56, PCR analysis of transgenic rice plants. The genomic DNA isolated from rice plants were PCR amplified using specific primers for the inserted gene. The specific bands were identified on 1% agarose gel compared with non-transgenic rice plant.

FIG. 57. Activity staining of starch synthase on renaturing SDS-PAGE gel with Iodine solution. The positive staining of maize SSI-2 indicated the expression of maize SSI-2 in transgenic rice plants.

FIG. 58. ***SSI-1, SSI-2, and SSI-3 construct diagram.*** Three forms of SSI were constructed in the pET expression system (see Methods). pExs10a encodes SSI-1, the full length maize SSI (583 amino acids). pExs8 encodes a truncated SSI, SSI-2, with amino acids #8-52 deleted from the N-terminus of SSI-1. pExs1d encodes the most truncated form of SSI, SSI-3, with the first 93 amino acids deleted from SSI-1. A depiction of the *waxy* gene, encoding GBSS, is also included for comparison. The amino acid motif KS/TGGL, the putative binding site for ADPGlc, is indicated by the triangles. The KS/TGGL motif is located 18 amino acids from the N-terminus in GBSS, while the motif is 106 amino acids from the N-terminus in maize SSI. Drawing not to scale.

FIG. 59. *SSIIa-1 and SSIIa-2 construct diagram.* Two forms of SSIIa were constructed in the pET expression system. pExs3c encodes SSIIb-1, the putative full length maize SSIIb. N-terminal sequencing of SSIIa-1 revealed that the polypeptide chain started at amino acid #1, so the length of SSIIa-1 is 669 amino acids. pExs3a encodes a truncated form of SSIIa, SSIIa-2, with the first 176 N-terminal amino acids deleted from SSIIa (493 amino acids total). A depiction of the *waxy* gene, encoding GBSS, is also included for comparison. The amino acid motif KTGGL, the putative binding site for ADPGlc, is indicated by the triangles. The

KTGGL motif is located 18 amino acids from the N-terminus in GBSS, while the motif is 194 amino acids from the N-terminus in maize SSIIa.

FIG. 60. *SSIIb-1 and SSIIb-2 construct diagram*. Two forms of SSIIb were constructed in the pET expression system (see Methods). pExs9 encodes SSIIb-1, the putative full length maize SSIIb. N-terminal sequencing of SSIIb-1 revealed that the polypeptide chain started at amino acid #1, so the length of SSIIb-1 is 637 amino acids. pExs9a encodes a truncated form of SSIIb, SSIIb-2, with the first 144 N-terminal amino acids deleted from SSIIb (492 amino acids total). A depiction of the *waxy* gene, encoding GBSS, is also included for comparison. The amino acid motif KTGGL, the putative binding site for ADPGlc, is indicated by the triangles. The KTGGL motif is located 18 amino acids from the N-terminus in GBSS, while the motif is 158 amino acids from the N-terminus in maize SSIIb.

FIG. 61. ***Temperature Curves for SSI enzymes.*** All assay components, except enzyme and [U-^{14}C]-ADPGlc, were mixed and then preincubated at each temperature for 3 minutes before addition of enzyme and ADPGlc. For all assays, the final concentration of [U- ^{14}C]-ADPGlc was 3 mM, while amylopectin was 6 mg/ml. Each point is an average of three separate determinations.

FIG.62. *Temperature Optima of SSIIa-1 and SSIIa-2*. All assay components, except enzyme and [U-^{14}C]-ADPGlc, were mixed and then preincubated at each temperature for 3 minutes before addition of enzyme and ADPGlc. For assays in the presence of 0.5 M citrate, 5 mg/ml amylopectin was used as primer. For assays without citrate, 10 mg/ml amylopectin was used. For all assays, the concentration of [U-^{14}C]-ADPGlc was 3 mM. Each point is an average of three separate determinations.

FIG. 63. *Temperature Optima of SSIIb-1 and SSIIb-2*. All assay components, except enzyme and [U-^{14}C]ADPGlc, were mixed and then preincubated at each temperature for 3 minutes before addition of enzyme and ADPGlc. For all assays, the concentration of [U-^{14}C]ADPGlc was 3 mM and the concentration of glycogen was 40 mg/ml. Each point is an average of three separate determinations.

PREFERRED EMBODIMENT -- DESCRIPTION

Gene shall mean the entire gene sequence or any mutations or varieties of the codon that produce the desired activity in the host or alternatively the section or sections of the gene sequence necessary to produce the desired activity in the host. For example glgC gene shall mean glgC$_{16}$, glgC$_3$ and other mutants that produce the desired activity in the host. ***Starch synthase gene shall mean full length SS, N-terminally-truncated SS or mutated SS with starch synthase activity.***

Glycogen like-shall mean polysaccharide material such as those produced as the main starch product by E.coli in its native state and by the hosts as taught in the above described paper by Hanping Guan.

Non Glycogen like- shall mean polysaccharide material which is plant like and is not produced as the main starch product by E.coli in its native state and by the hosts as taught in the above described paper by Hanping Guan.

Plant like starch- is non glycogen like.

Transformed gene-shall mean a gene that was somewhere in the lineage of the plant or bacteria introduced into the plant by means other than nature. Thus the progeny of a transformed host would continue to contain a transformed gene.

Transformed host- shall mean any organism containing one or more of the novel plasmids and/or a novel combination of starch synthetic genes discussed herein.

Within this application a number of different protocols have been employed to designate the same gene or synthase. MSS#=maize soluble starch synthase, SS#will likewise mean starch synthase though not necessarily maize. STS#will also designate soluble starch synthase. GBSS=granule bound starch synthase. SBE#=starch branching enzyme, MBE= maize starch branching enzyme, MSBE#=maize starch branching enzyme, and BE#=starch branching enzyme.

The present invention broadly encompasses transforming hosts such as bacteria or plants with plant starch synthetic genes that produce a non glycogen like material (a bacteria containing BEI and BEII from maize produces a glycogen like material). Starch bearing plants and organisms hereinafter are referred to as the host. One of the primary aspects of this invention is the generation of plant like starch from a bacterial host and the production of altered starch in a plant host. The present invention has been exemplified in both bacteria and in transformed rice plants. The host can contain though it is not a limitation, an unlimited supply of ADPG from the addition of the glgC gene (the bacterial gene) to the plant. Additionally the present invention encompasses plasmids that contain the maize genes and/or the bacterial genes in a construct adapted for use in a bacteria and constructs adapted for use in a plant. The plasmids in the plant construct preferably containing an active promoter recognized by the plant, a transit peptide, and the cleavage site that permits the protein to cleave from the transit peptide when crossing into the amyloplast in the plant. The plasmids used in the rice transformation specifically encompassed the maize 10 kd zein promoter, and the transit peptide from the maize SSI gene in the constructs adapted for plant use. The present invention also encompasses the plant producing the altered starch in the starch storage section of the plant or within the host cell and the altered starch itself. Additionally the present invention encompasses the combination of a number of starch genes in combination being active in a host such that the host produces differing non glycogen polysaccharides. Still further the present invention encompasses a method of making plant like starch in a bacterial host and the method of making altered plant like starch (altered in relationship to the type or amount of starch that the host makes without the constructs containing the genes), in a plant. Yet another object of the present invention is the addition of a gene that encodes for the substrate ADPG used to form starch.

The present invention encompasses a plasmid or combination of plasmids in the same host having a promoter adapted for use in a plant and a gene encoding for ADPGlc Pyrophosphoroylase, preferably a bacterial gene, and a gene encoding for starch synthase I or its mutant form. The present invention also encompasses the combination of a promoter adapted for use in a plant and optionally a gene encoding for ADPGlc Pyrophosphoroylase, preferably a bacterial gene, and a gene encoding for starch synthase I or its mutant form, and at

least one gene encoding for branching enzyme transformed into a plant host.

The present invention encompasses a plasmid or combination of plasmids in the same host having a promoter adapted for use in a plant and a gene encoding for ADPGlc pyrophosphorylase, preferably a bacterial gene, and a gene encoding for starch synthase Iia or its mutant form. The present invention also encompasses the combination of a promoter adapted for use in a plant and optionally a gene encoding for ADPGlc pyrophosphorylase, and a gene encoding for starch synthase Iia or its mutant form, and at least one gene encoding for branching enzyme transformed in to a plant host.

The present invention encompasses a plasmid having a promoter adapted for use in a plant and a gene encoding for ADPGlc pyrophosphorylase, preferably a bacterial gene, and a gene encoding for starch synthase IIb and its mutant form. The present invention also encompasses the combination for a promoter adapted of use in a plant and an optional gene encoding for ADPGlc pyrophosphorylase, and a gene encoding for starch synthase IIb or its mutant form and at least one gene encoding for branching enzyme transformed in to a plant host.

The present invention encompasses a plasmid having a promoter adapted for use in a plant and a gene encoding for Pyrophosphoroylase, preferably a bacterial gene, and genes encoding for at least one of the following genes starch synthase I, starch synthase IIa, starch synthase IIb, DU1. The present invention also encompasses the combination of a promoter adapted for use in a plant and a gene encoding for ADPGlc Pyrophosphoroylase, preferably a bacterial gene, and genes encoding for at least one of the following genes starch synthase I, starch synthase IIa, starch synthase Iib and DU1, , and at least one gene encoding for branching enzyme transformed in to a plant host.

The present invention encompasses a plasmid or combination of plasmids in the same host having a promoter adapted for use in a plant and a gene encoding for ADPGlc Pyrophosphoroylase, preferably a bacterial gene, and genes encoding for at least one of the following genes starch synthase I, starch synthase IIa, IIb and starch synthase III (DU1). The present invention also encompasses the combination of a promoter adapted for use in a plant

and an optional gene encoding for ADPGlc pyrophosphorylase, preferably a bacterial gene, and genes encoding for at least one of the following genes starch synthase I, starch synthase IIa, IIb starch synthase III(DU1), and at least one gene encoding for branching enzyme, and at least one gene encoding for the debranching enzyme transformed in to a plant host.

The present invention encompasses a plasmid or combination of plasmids in the host having a promoter adapted for use in a bacteria or yeast and a gene encoding for ADPGlc Pyrophosphoroylase, preferably a bacterial gene, and a gene encoding for starch synthase I. The present invention also encompasses the combination of a promoter adapted for use in a bacteria or yeast and a gene encoding for ADPGlc Pyrophosphoroylase, preferably a bacterial gene, and a gene encoding for starch synthase I, and at least one gene encoding for branching enzyme transformed in to a bacteria or yeast host.

The present invention encompasses a plasmid or combination of plasmids in the host having a promoter adapted for use in a bacteria or yeast and a gene encoding for Pyrophosphoroylase, preferably a bacterial gene, and a gene encoding for starch synthase IIa. The present invention also encompasses the combination of a promoter adapted for use in a bacteria or yeast and optionally a gene encoding for ADPGlc Pyrophosphoroylase, preferably a bacterial gene, and a gene encoding for starch synthase IIa, and at least one gene encoding for branching enzyme transformed in to a bacteria or yeast host.

The present invention encompasses a plasmid or combination of plasmids in the same host having a promoter adapted for use in a bacteria or yeast, and a maize gene encoding for starch synthase III(DU1). The present invention also encompasses the combination of a promoter adapted for use in a bacteria or yeast and an optional gene encoding for ADPGlc Pyrophosphoroylase, preferably a bacterial gene, and a gene encoding for starch synthase III, and at least one gene encoding for branching enzyme transformed in to a bacteria or yeast host.

The present invention encompasses a plasmid or combination of plasmids in the same host having a promoter adapted for use in bacteria or in yeast and a gene, and genes encoding for at least one of the following genes starch synthase I, starch synthase IIa,IIb, starch synthase III(DU1). The present invention also encompasses the combination of a promoter adapted for

use in bacteria or in yeast and a gene encoding for ADPGlc Pyrophosphoroylase, preferably a bacterial gene, and genes encoding for at least one of the following genes starch synthase I, starch synthase IIa,IIb, starch synthase III, and at least one gene encoding for branching enzyme transformed in to bacteria or into yeast hosts.

The present invention encompasses a plasmid or combination of plasmids in the same host having a promoter adapted for use in bacteria or in yeast and a gene encoding for ADPGlc Pyrophosphoroylase, preferably a bacterial gene, and genes encoding for at least one of the following genes starch synthase I, starch synthase IIa,IIb, starch synthase III The present invention also encompasses the combination of a promoter adapted for use in bacteria or in yeast and a gene encoding for Pyrophosphoroylase, preferably a bacterial gene, and genes encoding for at least one of the following genes starch synthase I, starch synthase IIa,IIb, starch synthase III(DU1), and at least one gene encoding for branching enzyme, and at least one gene encoding for the debranching enzyme transformed in to a bacteria or into a yeast host.

The present invention encompasses the truncated versions of the SSI and the SSII and the SSIII genes that still provide protein that is sufficient to make the polysaccharide.
By transforming different combinations of SS and SBE into *E. coli* HPG204(DE3) or G6MD3 defective in GS and GBE, we obtained the first evidence that maize SSI, SSII and SSIII have different specificities in the size of glucans synthesized see fig one.. Herein, we present the model system to produce differing polysaccharides from hosts with SS and SBE in *E.coli* by metabolic engineering. We also demonstrated that the truncated forms of SS had different Vmax, temperature stability and kinetic properties (Table, Fig).

We also demonstrated that transformation of starch synthase and/or branching enzyme in *E .coli* resulted in production of polysaccharides differing in size and structure. These polysaccharides can be used in food and nonfood industries to replace and/or complement starch functionalities. A large amount of these polysaccharides can be produced with fermentation technology.

Starch biosynthesis in higher plants and glycogen biosynthesis in *E. coli* have similar reactions which use adenosine diphosphate glucose (ADPGlc) as a substrate. This similarity

allows us to use plant starch synthase (SS) and starch branching enzyme (SBE) to complement the functions of glycogen synthase (GS) and glycogen branching enzyme (GBE) in *E. coli* G6MD3, which is deficient in GS and GBE. Transformation of *E.coli* glgC gene and maize starch synthase gene in *E.coli* G6MD3 produced linear a 1,4 glucan similar to amylose. coexpression of the glgC, maize starch synthase and maize branching enzyme produced branched polysaccharides. However, distinct properties of plant starch branching enzyme and starch synthase make it possible to synthesize different polysaccharides in *E.coli.* While maize SSI preferentially synthesis short chains (dp 6 - 15), SSII and SSIII preferentially transferred long chains (dp > 24) and intermediate chains (dp 16 - 24) respectively. Transformation of different maize starch synthases, *E.* coli glycogen synthase (glgA) and/or maize branching enzymes into *E.coli* HPG96 or *E. coli* G6MD3 resulted in the synthesis of different sizes of polysaccharide with DP 500-4000.These polysaccharides synthesized in *E .coli* by maize SS have different physical-chemical properties than polysaccharides synthesized in natural organisms including starch from plant sources and glycogen from animals. The polysaccharide can be used in food and nonfood industries to replace and/ or complement starch functionalities. A large amount of these polysaccharide can be produced by fermentation technology. The following materials were employed in the construction of the present invention some of the starting material are commercially available from Novagen in Madision Wisconsin ET-23d(+) DNA under catalog number 69748-1 and BL21(DE3) under catalog number 69387-1; ET-21a(+) DNA under catalog number 697401.

Plant Hosts

The following plasmids have been transformed into rice plants Transgenic 1, MSTSIA(pExs52) and glgC$_3$ (pExs66), MSTSIIa and glgC$_3$ (pExs53 and pExs56). The second group of rice transformatns contain MSTSIIc and glgC$_3$ (pExs54 and pExs56).The third group of transformation: transgenic 5 MSTSIII and glgC$_3$ (pExs 61 and pExs 66); transgenic 6 Mwx glgC$_3$ p-Exs65 and pExs66). Generally see figures 25-41 for plasmid maps and figure 43-55 for sequences used in the plasmid. Additionally, glgA and glgB and glgC were combined and transformed into rice. This is combining the rice plants starch pathway with the gene encoding for ADPG and the genes encoding for at least one of the following enzymes, SSI, SSII, SSIII, Debranching enzymes, BEI, BEII, GBSS (wx).

These plasmids could have been transformed into other cereals such as corn, wheat, barley, oats, sorghum, milo in substantially the plasmid that is shown in the figures for the plant host. The promoter could be the waxy gene which is published, other additional zein promoters are known and could be used as the promoter. The promoter used herein is described in Figures 45aand 45b.

Additionally these plasmid with little additional work could be transformed into dicots such as potatoes, sweet potato, taro, yam, lotus cassava, peanuts, peas, soybean, beans, chickpeas. The promoter could be selected to target the starch storage area of the particular dicots (some are roots some are tubers). Various method of transforming monocots and dicots are known in the industry and the method of transforming the genes is not critical to the present invention. The plasmid can be introduced into Agrobacterium tumefaciens by the freeze-thaw method of An et al.(1988) Binary vectors. In Plant Molecular Biology Manual A3, S.B. Gelvin and R.A. Schilperoot,eds (Dordrecht, The Netherlands: Kluwer Academic Publishers), pp. 1-19. Preparation of Agrobacteruim inoculum carrying the construct and inoculation of plant material , regeneration of shoots, and rooting of shoots are described in Edwards et al. (1995). Biochemical and molecular characterization of a novel starch synthase from potatoes. Plant J. 8, 283-294. Additionally promoters for different dicots are known particularly 35sCaMV and Monsanto has also published a promoter that is useful in potatoes called a patatin promoter.

A number of monocots are also starch bearing plants but until about a decade ago monocots were difficult to develop transformants. The most prominent methods of transformation presently used in monocots is the gunning of micro projectiles into the plants or using Agrobacterium and subsequent regeneration of the plants from the transformed materials. Various tissues and cells can now be transformed with plasmids into monocot hosts. In fact there are teaching from at least five ago on methods of transforming not only callus but also cotyledons. The methods of transforming plants and selecting for the transformants with either selectable or screen able markers are also well known. The use of the marker in the same plasmid and the use of the markers in a separate plasmid that is co transformed into the host are well known in the art by those of ordinary skill in the art. The biotechnology methods of forming plasmids and transforming plants are listed in the book entitled AShort Protocols In Molecular Biology@, 3rd ed. Published in 1995 by JOHN WILEY& Sons, Inc. Additionally ,

methods of transforming with the gun and with protoplasts are taught in a number of issued patents to Dekalb and Agracetus and Ciba.

PREFERRED EMBODIMENT - OPERATION

EXAMPLE 1

Construction of the *E. coli* expression vector.

The expression vector pExs2 was derived from pET-23d (Novagen) and pGP1-2 (15). The expression vectors pExs-trc and pExs-trc3 were derived from pTrc99a (Pharmacia) and pGP1-2. The BglII/PstI fragment (2192 bp) containing the pBR322 origin of replication was deleted from pET-23d and replaced with the BamHI/PstI fragment (3 kb) containing origin p15A and kanamycin resistance gene from pGP1-2. This process generated plasmid pEXS1 containing both ampicillin and kanamycin resistance genes. The ampicillin resistance gene was inactivated by deletion of the ScaI / BglI fragment (360 bp, BglI end was filled in and blunt-end ligated with ScaI end). Inactivation of the ampicillin resistance gene in pEXS1 generated the expression plasmid pEXS2, containing the T7 promoter, T7 terminator, kanamycin resistance gene and p15A origin of replication. Plasmid pTrc99a was digested with Nde1, filled in with kelnow fragment and blunt-end ligated to remove NdeI site. A Nde1 site was introduced at the NcoI site by mutagenesis to generate plasmid pExs-trc. The BglI and PvuII fragment(2.48 kb) in pExs-trc containing the pBR322 origin of replication was replaced by BglI/BamHI (filled in with Klenow fragment) fragment (3 kb) containing origin p15A and kanamycin resistance gene from pGP1-2 to generate pExs-trc2. The ampicillin resistance gene was inactivated by deletion of the ScaI / BglI fragment (360 bp, BglI end was filled in and blunt-end ligated with ScaI end). Inactivation of the ampicillin resistance gene in pExs-trc2 generated the expression plasmid pExs-trc3.

Construction of expression plasmids for maize SS. For expression of maize SS in *E. coli*, The PCR method was used to modify the N-terminus of maize SS using the following nucleiotides: primer Exs4 (5'-CAAGAATGCTGCGGGAGTC-3'), primer Exs23 (5'-AAGTCGACATATGTGCGTCGCGGAGCTGAGCAG-3'), primer Exs 57 (5'-GGGCCCCATATGAGCATTGTCTTTGTAACCGG-3'), primer Exs1 (5'-

CTCGGGCCCATATGGGGGAGAATGTTATGAA-3'), primer Exs2 (5'-GAGGCATCAATGAACACAAAGTCG-3'), Primer Exs33 (5'-GAAGGGCCCCATATGGCTGAGGCTGAGGCCGGGGGCAAG-3'), primer Exs16 (5'-TTGGATCCATATGGGAGCTGCGGTTGCATTGGG-3') and primer Exs17 (5'-CCTGCGGGCTCTGGCTTCACC), primer Exs 55 (5'-TTGGATCCATATGAACGTCGTCGTGGTGGCTTC-3'), primer 56 (5'-GCATACCATGGAACCTCAACAGC-3'), primer 53 (5'-GGTACCATATGAACGTCGTCTTCGGCG-3'), primer Exs 54 (5'-GACAGGCCCGTAGATCTTCTCC-3'), primer Exs -wx (5'-TTGGTACCATATGGCCAGCGCCGCCGGCATGAACG-3'). Primer Exs 4 paired respectively with primer Exs23 and Exs 57 was to modify the N-terminus of maize SSSI gene to generate pExs-10 and pExs-1d. Primer Exs2 paired individually with primer Exs33 and Exs1 was to modify the N-terminus of maize SSSII to generate pExs3c and pExs3a. Primer Exs17 paired individually with primer Exs16 and Exs55 was to modify the N-terminus of maize SSSIII to generate pExs-9 and pExs-9a. Primer Exs54 paired individually with primer Exs-wx and Exs53 was used to modify the N-terminus of maize GBSS to generate pExs-wx and pExs-wx2. The modified N-terminus was recombined with the rest of the SS gene in pBluescript SK plasmid. The reconstructed of maize SS was subcloned from pBluescript SK to the NdeI/NotI sites of the expression vector pET-21a (Novagen) , pExs-trc, pExs-trc3 (maps are attached, Table I shows the N-terminal sequence of SSS).

EXAMPLE 2

Construction of expression plasmids for *E. coli* ADPGlc pyrophosphorylase, BE and maize SBE.

E. coli glgB gene was excised from plasmid pOP12 (16). The BstX1 (filled in) / HindII fragment containing the glgB ribosome binding site and the full length glgB gene was cloned at the SmaI site of pBluescriptSK- (Stratagene). The glgB gene in pBluscriptSK- was subsequently cloned into pEXS2 at the XbaI / SalI sites to generate plasmid pEXSB. Primer G (5'- GAAGATCTGGCAGGGACCTGCACAC-3') and primer H (5'-GGACTAGTGCATTATCGCTCCTGTTTAT-3') were used to PCR the *E. coli* glgC gene coding for ADPGlc pyrophosphorylase from plasmid pOP12. A BglII site and a SpeI site introduced by PCR to the N-terminal and C-terminal site respectively, were used to clone the

PCR product into pBluscript SK- at the BamHI and SpeI sites. The glgC gene including its own ribosome binding site was subcloned into expression plasmid pEXS2 at the XbaI (filled in with Klenow fragment) and NotI site to generate plasmid pEXSc. The genes coding for mature maize SBEI and SBEII along with a ribosome binding site were subcloned from plasmids pET-23d-SBEI and pET-23d-SBEII into the plasmid pEXSc at the SpeI site to form the plasmids pEXSc-SBEI and pEXSc-SBEII. The gene coding for mature maize SBEII including a ribosome binding site was cloned into pEXSc-SBEI at the XbaI/NotI sites to form plasmid pEXSc-SBEI-SBEII. E.coli glgc gene and genes encoding maize SBEI and SBEII were also cloned in plasmid pExs-trc and pExs-trc3 respectively and together as described for pExs2.

EXAMPLE 3

Isolation of *E. coli* HPG204 deficient in GBE and GS activities

Homologous recombination was used for the strain construction. This was done according to the method described by Hamilton et al (Journal of Bacteriology, 1989, 171:4617-4622.) A temperature-sensitive pSC101 replicon was used to facilitate the selection. The gene coding for spectinomycin adenyltransferase was inserted at PvuII sites in plasmid pOP12 to form plasmid HPG9 which has spectinomycin resistance and has C-terminus of glgB gene and N-terminus of glgA gene deleted. The DNA fragment B=SA= with Spectinomycin resistant gene inserted between partial truncated glgB and glgA was subcloned into plasmid pMAK705 at XbaI site containing temperature sensitive replicon (Hamilton et al. Journal of Bacteriology, 1989, 171:4617-4622.) to form Plasmid pMak705B=SA=. Plasmid pMak705B=SA= was transformed into *TG1 cell.* After the transformed cell was cultured in 3 mL LB with 100 mg/mL Spectinomycin at room temperature overnight, the cells were plated on LB agar plate containing 100 mg/mL spectinomycin and incubated at 44^{0}C overnight. Single colonies were inoculated on LB agar plate containing 100 mg/mL spectinomycin and 0.2% glucose and incubated at 44^{0}C and at 37^{0}C overnight. The colonies at 37^{0}C were stained with iodine. The colony with negative staining was selected and grown in 100 mL LB at 37^{0}C overnight. The cells were harvested and homogenized in an extraction buffer for assaying glycogen synthase and branching enzyme activities. The cell lacking glgA and glgB activities was named as HPG204 [F=traD36 LacI^q D(glgBXCA) D(LacZ)M15proA$^+$B$^+$/SupED (hsdM-mcrB)5(r_k^- m_k^- McrB$^-$)thiD(lac-proAB),Spectinomycin^R, Chloramphenicol^R] .The lDE3

lysogenization kit fromNovagen was used for site specific integration of lDE3 prophage into *E. coli* HPG204 to form *E. coli* HPG204(DE3)[was DThe lysate was prepared with P1*vir* and its transduction into *E. coli* BL21 (DE3) [F=traD36 LacI^q D(glgBXCA) D(LacZ)M15proA$^+$B$^+$/SupED (hsdM-mcrB)5(r_k^- m_k^- McrB$^-$)thiD(lac-proAB),Spectinomycin^R, Chloramphenicol^R]

EXAMPLE 4

Expression of maize SS and SBE in *E. coli.*

Plasmid pExs-2 and pExs-trc3 has kanamycin resistance and p15A origin of replication. It is compatible with plasmid pET21a, pExs-trc, pTrc99A containing pBR322 origin. Expression plasmids pExs-2 and pET-21a were used to express SS and SBE in *E. coli* HPG204(DE3). Expression plasmids pExs-trc and pExs-trc3 were used for expression in *E. coli* G6MD3. This made it possible to transform different combinations of maize SS and SBE in *E. coli* HPG204(DE3), or G6MD3 which is deficient in GS and GBE activity. An overnight culture of cells transformed with maize SS and SBE was diluted 1:20 (v/v) in fresh LB containing 0.2 % glucose, 100 mg/mL ampicillin and 50 mg/mL kanamycin. The cells were grown at 37^{0}C for about 2 h to A600nm =0.6 before the expression of maize SBE and/or SS was induced by adding isopropyl b-D-thiogalactoside to 0.5 mM. Following growth at 25^{0}C for 4 h, the cells were harvested in a refrigerated centrifuge.

EXAMPLE 5

Isolation of highly branched a-glucan from *E. coli.*

Cell pellet (30 g) was resuspended and lysed by sonication in 150 mL 50 mM tris-acetate buffer (pH 7.5) containing 10 mM EDTA and 5 mM DTT. After a fraction of the homogenate was saved for assaying the STS and SBE activities, the homogenate was centrifuged at 20, 000*g* for 50 min at 4^{0}C. After collecting the supernatant, the pellet was resuspended in 150 mL water and boiled for 15 min with occasional stirring. The resuspension was centrifuged at 20, 000g for 30 at room temperature. After collecting the supernatant, the pellet was washed again with 100 mL water as above. 0.1 volumes of 50% Trichloric acid (TCA) were added to the pooled fractions. After storing on ice for 30 min, the precipitate was spun down at 15,000g for 20 min, then washed with 30 mL 5% TCA and centrifuged as above.

The supernatant and wash were pooled and one volume of absolute ethanol was added. After storing on ice for 30 min, the polysaccharide was collected by centrifuging at 15, 000g for 15 min. The polysaccharide was redissolved in water and precipitated with ethanol. This step was repeated twice. The pellet was washed with methanol twice, acetone twice and dried over silica gel at room temperature.

EXAMPLE 6

Isolation of linear a 1,4 polysaccharide from *E. coli*

Resuspend 50 grams of cell pellet in 250 mL of 50 mM Tris acetate buffer, pH 7.5, containing 10 mM EDTA and 5 mM DTT. Sonicate for 3 minutes (45 seconds/time, output # 8, repeat 4 times with 30 seconds interval). The homogenate is centrifuged at 12,000 rpm (SA1500) for 50 minutes. The supernatant is checked with iodine staining and discarded. (Same 1 mL homogenate and 1 mL supernatant for enzyme assay. The pellet is resuspended & extracted in 100 mL DMSO. Extract the polysaccharide by heating and stirring in boiling water bath for 15 min. Let it cool down to below 40 ^{0}C and centrifuge at 12,000 rpm for 30 min at room temperature. The supernatant is pooled. The pellet is extracted two more times with 100 mL DMSO. Equal volume of absolute ethanol is added into the pooled supernatant, mixed and stored on ice for 30 minutes. Centrifuge at 12,000 rpm for 30 min at 4 ^{0}C . The pellet is redissolved in 20 mL DMSO by heating in boiling water bath. 80 mL water is added and mixed well. After adding 10 mL butanol to the solution, the solution is mixed and stored at 0 ^{0}C for one hr (mix once a while). Centrifuge at 12000 rpm for 30 min at 4^{0}C. Repeat the step once. The pellet is redissolved in 90 mL hot water by heating in boiling water bath. Insoluble materials are immediately removed by centrifugation at room temperature. Add 10 mL butanol to the supernatant, stay at 0 ^{0}C for 1 hr and centrifuge at 12000 rpm for 30 min at 4 ^{0}C. Repeat the step once. The amylose precipitate is redissolved in 90 mL hot water by heating, and 10 mL butanol are added to the solution. After storing at 40 ^{0}C on ice for one hour, it is centrifuged at 4 ^{0}C for 30 min. Repeat the step once. The pellet is redissolved in 100 mL 10% butanol by heating. The amylose is stored at 0 ^{0}C and precipitated by centrifuging at 12000 rpm for 30 min at 4 ^{0}C. The pellet is washed with 25 mL methanol 3 times and with acetone once. Dry over silica gel.

EXAMPLE 7

Enzyme assays

5 mL of supernatant were used to assay STS and SBE activities as previously described (Preiss) with minor modification. The reaction mixture for STS contained 100 mM Bicine buffer, 10 mg/mL glycogen, 0.5 mg/mL BSA, 0.5 M sodium citrate, 25 mM potassium acetate, 10 mM GSH, 3 mM [14C]ADPGlc (500 dpm/nmol) and enzyme in a final volume of 0.1 mL. The reaction was carried out at 25^{0}C for 15 min and terminated by boiling for 2 min. The unincorporated [14C]ADPGlc was separated with Dowex anion exchange column (200-400 mesh, Sigma Chemical Co.). One unit of activity is defined as 1 nmol Glc incorporated into the a-glucan per min at 25^{0}C. SBE activity was determined by phosphorylase stimulation assay. One unit of activity is defined as 1 mmol Glc incorporated into the a-glucan per min at 30^{0}C.

Example 8

Enzyme purification

For the recombinant SS purification, the cell pellet was resuspended in sonication buffer (50 mM Tris-acetate, pH 7.5, 10 mM EDTA, and 5 mM DTT; 7 ml buffer per gram of cell mass), and cells were lysed using a Fisher 550 Sonic Dismembrator with 5 x 1 min. bursts with 30 sec. intervals. The homogenate was centrifuged at 9600g for 30 minutes. SSI in the supernatant was then precipitated by slowly adding neutralized saturated ammonium sulfate to 40% saturation. After stirring on ice for an additional 50 minutes, proteins were collected by centrifugation at 12700g for 45 minutes. The protein pellet was then redissolved in buffer A (50 mM Tris, pH 7.5, 1 mM EDTA, and 5 mM DTT) containing 0.1 M KCl and dialyzed against the same buffer, with one change of buffer. After dialysis, the sample was centrifuged at 13000g for 20 minutes to remove insoluble materials. The resulting supernatant was loaded onto an amylose affinity column pre-equilibrated with dialysis buffer, and the flow through was collected. The column was washed with 10 column volumes of buffer A containing 0.1 M KCl, and then with buffer A containing 0.5 M KCl and 0.5 M maltose, collecting fractions during both washes. The active fractions were pooled and dialyzed overnight against buffer A,

with one change of buffer. The next day, the amylose column sample was filtered and applied to a mono Q 5/5 FPLC column (Pharmacia). After washing with buffer A, a 20 ml 0-0.4 M KCl gradient was employed. The active fractions were electrophoresed on an 8% SDS-PAGE gel (31) to determine the purity of SSI in those fractions; the fractions which were apparently homogeneous were pooled and concentrated using a Centricon-30 spin column (Amicon).

Table 1. Expression of maize starch synthases in *Escherichia coli* BL21(DE3).

Plasmids	Maize starch synthase genes	N-terminus	Protein (mg/mL)	Specific Activities* (units/mg Protein)
pET21a	Native plasmid		1.8	0.009
pEXS-3a	SSIIa-2	GENVMNVIVV	2.8	0.069
pEXS-3c	SSIIa	AEAEAGGKD	2.8	0.28
pEXS-1d	SS1-3	MSIVFVTGEA	3.0	0.23
pEXS-8	SSI-2	GDLGLEPEG	1.9	0.097
pExs-10	SSI	CVAELSREG	1.2	0.043
pEXS-9	SSIIb	GSVGAALRSY	1.8	0.515
pEXS-9a	SSIIb-2	MNVVVVASEC	2.6	0.36
pEXS-wx	GBSS (waxy)	ASAGMNVVFV	2	0.033
pEXS-wx2	GBSS(2)	MNVVFVGAEM	2.2	0.32

* One unit activity is defined as one μmol glucose incorporated into a-1,4 glucan per minute at 25°C using 5 mg/mL glycogen as primer.

Table 2. Properties of polysaccharides synthesized in *E. coli*.

Plasmid	Protein (Mg/mL)	STS activity (u/mg protein)	BE activity (u/mg protein)	Imax (nm)	DP	CL	Yield (mg dry wt/g wet cell)
pExsCA				580	700	10.6	3.3
pExsC-9				585	1007	35.8	4.1
pExsC-3a	13.3	.0015		600	983	53	1.0
pExsC-8	12.6	.0032		580	435	31.8	7.4
pExsC-wx	15.2	0.002		600	836	15.6	9.1
pExsC-I-II + pExs9	7.84	0.08	4.71	480	2333	19	30
pExsC-I-II + pExs3a	13.61	0.011	1.56	530	3616	22	36
pExsC-I-II + pExs8	11.95	0.042	3.33	525	1689	17.5	131
pExsC-I-II + pExs10	8.9	.0094	3.65	500	3174	16.6	24.5
pExsC-I-II + pExswx	11.7	.007	5.4	450	2970	14.8	33.8
pExsC-I-II + pExsA1	11	0.13	4.48	475	3940	14	28.9

Table 3. Properties listed by degree of DP of polysaccharides synthesized in *E. coli*.

Plasmid	Protein (Mg/mL)	STS activity (u/mg protein)	BE activity (u/mg protein)	Imax (nm)	DP	CL	Yield (mg dry wt/g wet cell)
pExsC-I-II + pExsA1	11	0.13	4.48	475	3940	14	28.9
pExsC-I-II + pExs3a13.61		0.011	1.56	530	3616	22	36
pExsC-I-II + pExs108.9		.0094	3.65	500	3174	16.6	24.5
pExsC-I-II + pExswx11.7		.007	5.4	450	2970	14.8	33.8
pExsC-I-II + pExs9	7.84	0.08	4.71	480	2333	19	30
pExsC-I-II + pExs8	11.95	0.042	3.33	525	1689	17.5	131
pExsC-9				585	1007	35.8	4.1
pExsC-3a	13.3	.0015		600	983	53	1.0
pExsC-wx	15.2	0.002		600	836	15.6	9.1
pExsCA				580	700	10.6	3.3
pExsC-8	12.6	.0032		580	435	31.8	7.4

Table 4. Properties listed by degree of λmax of polysaccharides synthesized in *E. coli*.

Plasmid cell)	Protein (Mg/mL)	STS activity (u/mg protein)	BE activity (u/mg protein)	λmax (nm)	DP	CL	Yield (mg dry wt/g wet
pExsC-3a	13.3	.0015		600	983	53	1.0
pExsC-wx	15.2	0.002		600	836	15.6	9.1
pExsC-9				585	1007	35.8	4.1
pExsCA				580	700	10.6	3.3
pExsC-8	12.6	.0032		580	435	31.8	7.4
pExsC-I-II + pExs3a	13.61	0.011	1.56	530	3616	22	36
pExsC-I-II + pExs8	11.95	0.042	3.33	525	1689	17.5	131
pExsC-I-II + pExs10	8.9	.0094	3.65	500	3174	16.6	24.5
pExsC-I-II + pExs9	7.84	0.08	4.71	480	2333	19	30
pExsC-I-II + pExsA1	11	0.13	4.48	475	3940	14	28.9
pExsC-I-II + pExswx	11.7	.007	5.4	450	2970	14.8	33.8

Table 5. Properties listed by degree of CL of polysaccharides synthesized in *E. coli.*

Plasmid	Protein (Mg/mL)	STS activity (u/mg protein)	BE activity (u/mg protein)	λmax (nm)	DP	CL	Yield (mg dry wt/g wet cell)
pExsC-3a	13.3	.0015		600	983	53	1.0
pExsC-9				585	1007	35.8	4.1
pExsC-8	12.6	.0032		580	435	31.8	7.4
pExsC-I-II + pExs3a	13.61	0.011	1.56	530	3616	22	36
pExsC-I-II + pExs9	7.84	0.08	4.71	480	2333	19	30
pExsC-I-II + pExs8	11.95	0.042	3.33	525	1689	17.5	131
pExsC-I-II + pExs10	8.9	.0094	3.65	500	3174	16.6	24.5
pExsC-wx	15.2	0.002		600	836	15.6	9.1
pExsC-I-II + pExswx	11.7	.007	5.4	450	2970	14.8	33.8
pExsC-I-II + pExsA1	11	0.13	4.48	475	3940	14	28.9
pExsCA				580	700	10.6	3.3

Table 6. *Purification Tables for SSI-1, SSI-2, and SSI-3.*

SSI-1	volume (ml)	total mg protein	activity U/mg	total Units	purification (fold)
Homogenate	630	4347	0.018	76.2	1
Supernatant	570	2622	0.020	53.0	1.1
0-40% $(NH_4)_2SO_4$	48	494	0.058	28.7	3.2
Amylose column	17	2.6	5.03	11.3	279
monoQ column	0.27	0.26	12.2	3.2	677

SSI-2	volume (ml)	total mg protein	activity U/mg	total Units	purification (fold)
Homogenate	380	2797	0.0356	99.6	1
Supernatant	320	2118	0.0340	72.0	1
0-40% $(NH_4)_2SO_4$	48	466	0.133	61.8	3.7
Amylose column	17.5	1.2	22.6	26.5	634
monoQ column	1.0	0.325	17.2	5.6	483

SSI-3	volume (ml)	total mg protein	activity U/mg	total Units	purification (fold)
Homogenate	1300	16770	0.23	3900	1
Supernatant	1100	9790	0.31	3080	1.3
0-40% $(NH_4)_2SO_4$	237	2204	1.5	3294	6.5
Amylose column	63	30	22.4	668	97
monoQ column	3.6	3.1	30.5	93	132

Notes: Assays performed during the course of purification contained 10 mg/ml glycogen and 3 mM [U-^{14}C]-ADPGlc. Assays were performed at room temperature in the presence of 0.5 M citrate. 1 Unit = 1 µmol [U-^{14}C]-glucose transferred per min.

Table 7. *Primer Kinetics for SSI enzymes*

Amylopectin

		SSI-3	SSI-2	SSI-1
+ citrate	K_m	240 ± 45	230 ± 50	150 ± 40
	V_{max}	26.3 ± 0.5	33.4 ± 2.1	22.5 ± 0.6
- citrate	K_m	230 ± 60	68 ± 3	120 ± 20
	V_{max}	13.2 ± 0.3	9.94 ± 0.18	7.62 ± 0.99

Glycogen

		SSI-3	SSI-2	SSI-1
+ citrate	[a]V_{max}	43.4 ± 2.5	45.6 ± 3.3	39.0 ± 2.2
- citrate	[a]V_{max}	41.4 ± 2.9	45.5 ± 1.5	26.1 ± 1.4

Notes: Assays were performed at 37°C as described in the Materials and Methods. Data are expressed as the average of three independent determinations along with the standard deviation. K_m are expressed as µg/ml primer and V_{max} are in µmol/min/mg protein. ADPGlc = 3 mM in all assays.

[a]Because saturating glycogen concentrations could not be obtained, a standard 20 mg/ml glycogen was used to compare enzyme rates for that primer.

Table 8. ***ADPGlc Kinetics for STSI enzymes.*** Assays and data evaluation are as in Table II. K_m are expressed as mM ADPGlc and V_m are in µmol/min/mg protein. 5 mg/ml amylopectin was used as primer for all assays.

		STSI-3	STSI-2	STSI-1
+ citrate	K_m	0.33 ± 0.07	0.32 ± 0.02	0.18 ± 0.02
	V_m	26.4 ± 1.4	32.6 ± 0.8	18.0 ± 0.5
- citrate	K_m	0.62 ± 0.04	0.25 ± 0.04	0.24 ± 0.02
	V_m	14.7 ± 1.3	11.7 ± 0.7	6.38 ± 0.88

Table 9. ***Purification Tables for SSIIa enzymes.*** **Assays for SSIIa-2 purification contained 10 mg/ml glycogen and 1.5 mM [U-^{14}C]-ADPGlc (both are at saturating concentrations). Assays for SSIIa-1 purification contained 5 mg/ml amylopectin and 3 mM [U-^{14}C]-ADPGlc. Assays were performed at room temperature in the presence of 0.5 M citrate. 1 U = 1 μmol [U-^{14}C]-glucose transferred per min.**

SSIIa-2

	volume (ml)	total mg protein	activity U/mg	total Units	purification (fold)
Supernatant	300	1620	0.0216	34.8	1
0-40% $(NH_4)_2SO_4$	53	419	0.0606	25.4	2.8
Amylose column	20	9.3	0.991	9.3	45.9
monoQ column	0.9	0.94	4.81	4.5	222

SSIIa-1

	volume (ml)	total mg protein	activity U/mg	total Units	purification (fold)
Supernatant	335	2613	0.28	737	1
0-40% $(NH_4)_2SO_4$	47	427	0.96	409	3.4
Amylose column	25	11.5	8.04	92	28.7
monoQ column	1.0	4.8	9.10	44	32.5

Table 10. *Primer Kinetics for SSIIa enzymes.* Assays were performed as described in the Materials and Methods. Data are expressed as the average of three independent determinations along with the standard deviation. K_m are expressed in µg/ml and V_{max} are in µmol/min/mg protein. ADPGlc = 3 mM in all assays. *NA = not applicable; enzyme cannot be saturated by primer under these conditions.

Amylopectin

		SSIIa-2	SSIIa-1
+ citrate			
27°C	K_m	153 ± 22	182 ± 38
	V_{max}	7.82 ± 0.63	24.1 ± 0.5
37°C	K_m	133 ± 18	153 ± 64
	V_{max}	15.4 ± 0.6	41.1 ± 0.2
- citrate			
27°C	K_m	234 ± 30	404 ± 33
	V_{max}	4.31 ± 0.32	10.5 ± 0.3
37°C	K_m	1350 ± 220	-NA*
	V_{max}	7.84 ± 0.25	-NA*

Glycogen

		SSIIa-2	SSIIa-1
+ citrate			
27°C	K_m	50.7 ± 3.8	162 ± 17
	V_{max}	5.53 ± 0.44	14.2 ± 0.7
37°C	K_m	76.9 ± 7.8	350 ± 11
	V_{max}	11.3 ± 0.7	31.6 ± 0.8

Table 11. ***ADPGlc Kinetics for SSIIa enzymes.*** **Assays and data evaluations are as in Table II. Concentration of primer in each case was saturating for each enzyme and was determined by the experiments detailed in Table II. K_m are expressed as mM ADPGlc and V_{max} are in µmol/min/mg protein. *NA = not applicable, as the enzyme cannot be saturated by primer under these conditions.**

with amylopectin as primer

		SSIIa-2	SSIIa-1
+ citrate			
27°C	K_m	0.17 ± 0.04	0.48 ± 0.09
	V_{max}	4.83 ± 0.42	23.0 ± 2.5
37°C	K_m	0.28 ± 0.01	0.83 ± 0.08
	V_{max}	11.4 ± 0.6	49.1 ± 2.6
- citrate			
27°C	K_m	0.27 ± 0.02	0.46 ± 0.06
	V_{max}	4.87 ± 0.25	12.1 ± 0.8
37°C	K_m	0.28 ± 0.005	-NA*
	V_{max}	7.86 ± 0.53	-NA*

with glycogen as primer

with glycogen		SSIIa-2	SSIIa-1
+ citrate			
27°C	K_m	0.16 ± 0.03	0.19 ± 0.02
	V_{max}	4.41 ± 0.21	17.1 ± 0.7
37°C	K_m	0.15 ± 0.03	0.37 ± 0.04
	V_{max}	7.60 ± 0.94	40.1 ± 1.7

Table 12. ***Purification Tables for SSIIb-2 and SSIIb-1.*** **Assays performed during the course of purification contained 10 mg/ml glycogen and 3 mM [U-^{14}C]ADPGlc. Assays were performed at room temperature in the presence of 0.5 M citrate. 1 U = 1 μmol [U-^{14}C]glucose transferred per min.**

SSIIb-2	volume (ml)	total mg protein	activity U/mg	total Units	purification fold
Supernatant	890	9256	0.48	4450	1
0-40% $(NH_4)_2SO_4$	190	2660	1.24	3306	2.6
Amylose column	13	31.2	50.6	1573	105
monoQ column	6.6	16.3	56.8	939	118

SSIIb-1	volume (ml)	total mg protein	activity U/mg	total Units	purification fold
Supernatant	365	2336	0.64	1533	1
0-40% $(NH_4)_2SO_4$	56	436	2.35	1030	3.7
Amylose column	80	10.4	50.2	521	78
monoQ column	0.6	0.28	60.6	17.6	94

Table 13. *Kinetics for SSIIb enzymes.* Assays were performed at 37°C as described in the Materials and Methods. Data are expressed as the average of three independent determinations along with the standard deviation. For ADPGlc kinetics, K_m are expressed in mM ADPGlc. For primer kinetics, K_m are expressed as mg/ml primer, and 3 mM ADPGlc were used in the assays. V_{max} are in μmol $min^{-1}mg^{-1}$ protein.

ADPGlc Kinetics

		SSIIb-2	SSIIb-1
with glycogen			
	K_m	0.32 ± 0.04	0.71 ± 0.01
	V_{max}	130 ± 6	76.8 ± 3.2
with amylopectin			
	K_m	0.32 ± 0.03	0.40 ± 0.02
	V_{max}	90.9 ± 4.2	72.8 ± 2.8

Primer Kinetics

		SSIIb-2	SSIIb-1
glycogen			
	K_m	0.36 ± 0.02	0.43 ± 0.02
	V_{max}	120 ± 3	79.5 ± 3.3
amylopectin			
	K_m	0.26 ± 0.04	0.074 ± 0.008
	V_{max}	84.5 ± 2.4	67.9 ± 1.7

Table 14. *Comparison of kinetic data for expressed SS's.* Data for SSI and SSIIa are form Imparl-Radosevich *et al.*, 1998; Imparl-Radosevich J>, Li P, McKean Al, Keeling PL, and Guan HP, submitted for publication. K_m for amylopectin and glycogen are epxressed in mg/ml; K_m for ADPGlc are in mM and were determined in the presence of amylopectin and 0.5 M citrate. V_{max} are in μmol $min^{-1}mg^{-1}$. The K_m for glycogen for SSI could not be determined as saturating concentrations of glycogen could not be reached for this enzyme.

Kinetic parameter	SSI-3[a]	SSI-1	SSIIa-2[a]	SSIIa-1	SSIIb-2[a]	SSIIb-1
K_m for amylopectin	0.24	0.15	0.13	0.15	0.26	0.07
K_m for glycogen	—	—	0.077	0.35	0.36	0.43
K_m for ADPGlc	0.33	0.18	0.28	0.83	0.32	0.40
V_{max} (with amylopectin)	26.3	22.5	15.4	41.1	84.5	67.9
V_{max} (with glycogen)	43.4	39.0	11.3	31.6	120	79.5

[a]denotes N-terminally truncated form of SS, while any SS with the designation SS-1 is the full length version of the SS.

Table 15. The starch synthase activities of the chimerical enzymes. Generation of chimerical enzymes of maize starch synthase: the recombination of N-terminal extensions with C-terminal catalytic domains of starch synthase

The gene coding for N-terminal extensions of STSI, STSIIa and STSIIb were inserted, in the same (+) or reverse (-) orientation of original N-terminal DNA sequence, in front of the C-terminal catalytic domains of WX2, STSIIa and STSIIb, respectively. The chimerical enzymes were expressed in E.coli, and the activities were assayed.

	WX2 C-catalytic domain	STSIIa C-catalytic domain	STSIIb C-catalytic domain
STSI N-extension	**N1-WX2** (+) (-) NRA 9.0	**N1-C2** (+) (-) 6.6 39.7	**N1-C3** (+) (-) 89.2 NRA
STSIIa N-extension	**N2-WX2** (+) (-) 9.2 11.2	**N2-C2** (+) (-) 213.8 8.7	**N2-C3** (+) (-) 232.5 NRA
STSIIb N-extension	**N3-WX2** (+) (-) NRA NRA	**N3-C2** (+) (-) 11.2 NRA	**N3-C3** (+) (-) 400.5 12.0

N1: STSI N-terminal extension;
N2: STSIIa N-terminal extension;
N3: STSIIb N-terminal extension;
WX2: WX2 C-terminal catalytic domain;
C2: STSIIa C-terminal catalytic domain;
C3: STSIIb C-terminal catalytic domain.
(+): the N-terminal extensions were inserted in front of the C-terminal catalytic domains in same orientation;
(-): the N-terminal extensions were inserted in front of the C-terminal catalytic domains in reverse orientation.
* Starch synthase enzyme activity: nmol/min mgprotein.
* The residue glycogen synthase activity of BL21(DE3) is 2.6nmol/min mg protein.
* NRA--No recombinant available.

The photographs listed in the figures 42 and 21attempt to show the visual differences that are present into the starches as compared to those known in the art.

Description of the starch

Corn starch is a milky , slightly thickened gel which is slightly if at all flowable.

Rice starch forms two levels the upper level is a thickened syrup like consistency more flowable then corn starch (less thick then corn starch) opaque milky color (more translucent then corn starch in this level) and a lower level which is a very white glob not transmitting much light through this bottom level of material. This lower level is formed in a very thick mass and does not appear flowable.

Corn amylopectin is slightly less white then the top level of rice starch and is a very slightly opaque milky color (more translucent then corn starch) slightly less flowable then the rice top level.

Potato dextrin is the most transparent almost appearing clear but is still opaque white and it is very flowable appearing only slightly less flowable then water.

Waxy Maize starch will flow very slowly and has the consistency of honey. The color is very opaque transmitting little light and the color is only slightly less light then corn starch.

SSI starch made from plasmid pExs-8 has two distinct levels. The top level appears clear and slightly thicker then the flowability of water. The bottom level appears as a precipitate. This sample resembles the ornaments that contain little figures and plastic flakes resembling snowflakes. Like those ornaments when turned upside down the sample appears to be falling snow. However the flakes in this sample appear to be slightly gummy and appear in the first moments of level mixing to form a opaque white liquid.

SSI starch made from a host containing the following two plasmids pExsC BEI BEII and pExs8 is not as clear as the top level of pExs-8 and appears slightly less thick then pExs-8.

It has even more flowability then does Potato Dextrin.

SSIIb starch made from a host containing the following two plasmids pExsC BEI BEII and pExs-9 is not as clear as the top level of pExs-8 and appears slightly less thick then pExs-8. It has even more flowability then does Potato Dextrin.

WAXY starch made from a host containing the following two plasmids pExsC BEI BEII and pExs-wx is not as clear as the top level of pExs-8 but seems to have a few tiny thread like chains that settle to the bottom and when mixed give the material a slightly more white color and appears slightly less thick then pExs-8. It has even more flowability then does Potato Dextrin.

SSII starch made from a host containing the following two plasmids pExsC BEI BEII and pExs-3a is the color of corn starch and maybe slightly whiter but not as white as the bottom level of pExs8 and definitely transmitting more light through and has the flowability characteristic of pExs-8 when mixed.

glgA starch appears to have a very slight precipitate and is comparable in color to corn amylose pectin and ExsC BEI BEII and pExs-wx. And the flowability is between corn amylose and pExsC BEI BEII pExs-wx.

The samples of polysaccharides listed above form groups generally according to color as follows: waxy maize starch and corn starch and pExsC BEI BEII pExs3a and pExsc8 are the whitest group. The flowability characteristics of this group are fairly diverse. With corn starch a lump and Waxy maize starch only slightly flowable and pExsC BEI BEII and pExs-3a and pExsC-8 more like water then syrup. The second group contains corn amylopectin and pExsC BEI BEII pExs-wx and pExsC BEI BEII and pExs-A1 which are less white and clearer. The flowability of corn amylopectin is less then the other two members of this group but it is still similar. The last group is the least white and thus the clearest. This group includes pExsC BEI BEII and pExs-8, potato dextrin, pExsC BEI BEII and pExs-10, pExsC BEI BEII and pExs-9. The flowability of this group is also similar to each other.

Plant H sts

The following plasmids have been transformed into rice plants. The sequence for the mutant glgC gene is shown in Figure 46. The plasmids are made substantially in a similar manner as described above for the production of bacterial plasmid. Clearly the plasmid maps shown in figures 25-41 and this application and the listed short protocols allow the ordinarily skilled person in the art to make the present plasmids. The following combinations of plasmids have been transformed into rice plants. Additionally combinations of plasmids including the combination that includes all of the maize genes SSI, SSII, SSII, BEI, BEII, and GBSS in one host or alternatively in two host that are then crossed to form a hybrid having the entire complement of up regulated starch genes are being developed. Clearly the ordinarily skilled person in the art could have placed the sequences in the antisense positions to down regulate these genes to the extent that maize genes will down regulate the partial homologous rice genes. The first group of transgenic are group1, including rice transformants (transformed by microprojectile bombardment) containing MSTSI-2 (pExs52) and $glgC_3$ (pExs66), MSTSIIa-2 and $glgC_3$ (pExs53 and pExs56). The second group of rice transformants contains MSTSIIa and $glgC_3$ (pExs54 and pExs56). The third group of transformation contain: transgenic 5 MSTSIIb and $glgC_3$ (pExs 61 and pExs 66); transgenic 6 Maize wx and $glgC_3$ pExs65 and pExs66). Additionally, glgA and glgB and glgC are combined and transformed into rice. This last transformant is combining the rice plants starch pathway with the gene encoding for ADPG pyrophosphorylase and the bacterial genes. The combination of the plasmids encoding for at least one of the following enzymes, SSI, SSIIa, SSIIb, SSIII, Debranching enzymes, BEI, BEII, GBSS (wx)and some or all of the bacterial starch genes is also useful. There ae presently over 300 transformants in the greenhouse.The T1 transgenic rice plants have been screened and characterized (Figure 56, 57). 12 plants have successfully expressed maize SSI-2 in rice seeds. 21 plants have successfully expressed maize SSIIb in rice seeds. We are currently screening rice plants down regulated the rice SS expression by cosuppression and have 400 T2 plants in the greenhouse.

Maize Starch Synthase and its Mutant Forms.

In order to characterize the multiple forms of maize starch synthase, the genes coding for the full length SS and its N-terminally truncated forms were expressed in *E.coli*,. The recombinant enzymes were purified and kinetically characterized. We have demonstrated that different isoforms and its truncated forms all have distinct properties (Table 6-14, Figure 58-63). The specific activities (Vmax) of the purified maize SSI-1, SSI-2, and SSI-3 were 22.5, 33.4 and 26.3 :mol glc/min/mg of protein respectively, Our results have clearly indicated that the catalytic center of SSI is not located in its N-terminal extension. However, N-terminal truncation decreased the enzyme affinity for amylopectin, with the K_m for amylopectin of the truncated SSI-3 being about 60%-90% higher than that of the full length SSI-1. The effects of N-terminal truncation of SSIIa depend upon the assay conditions used. For both SSIIa-1 and SSIIa-2, the V_{max} of each enzyme increased 2-fold upon raising assay temperature from 27 ^{N}C to 37 ^{N}C (Tables II and III). However, the effect of temperature on ADPGlc affinity was different for SSIIa-1 and SSIIa-2. For the truncated SSIIa-2, the K_m for ADPGlc was not affected by raising temperature. In contrast, the K_m of ADPGlc for the putative full length SSIIa-1 increased 2 fold upon raising the assay temperature from 27 ^{N}C to 37 ^{N}C (Table III). Interestingly, the truncated SSIIa-2 exhibited a lower K_m for ADPGlc than SSIIa-1 did in all assay conditions used in this study except that they showed similar K_m values for ADPGlc when glycogen was used as a primer at 27EC. Although N-terminal truncation of SSIIa appears to lower the K_m for ADPGlc under most assay conditions, it also must be noted that the maximal velocity of the truncated SSIIa-2 is decreased by about 2-4 fold when compared to SSIIa-1. The truncated SSIIb-2 was found to be more temperature stable than the longer SSIIb-1 in the presence of citrate, while little difference was observed in their pH activity profiles. While the putative full length SSIIb-1 showed a similar V_{max} using amylopectin or glycogen as a primer, the N-terminally truncated SSIIb-2 showed a 40% increase in V_{max} using glycogen compared with amylopectin as a primer. N-terminal truncation of SSIIb increased its V_{max} by 25% with amylopectin as a primer. We also demonstrated that kimeric enzymes of maize starch synthase (combining the C-terminal domain of SS with different N-terminal sequences of SS or unrelated sequences would produce a functional enzyme with SS activity and altered properties) (Table 15).

Conclusions, Ramifications, and Scope

Accordingly, it can be seen that, according to the invention, The starch genes can produce new and altered starch in either host, plant or bacteria. Additionally, polysaccahrides very similar to corn starch can be produced in a bacterial host.

Although the description above contains many specificities, these should not be construed as limiting the scope of the invention but as merely providing illustrations of some of the presently preferred embodiments of this invention. Various other embodiments and ramifications are possible within it's scope. For example, different combinations of the plasmids in either host for the production of useful plant and useful grain and useful polysaccahrides.

Thus the scope of the invention should be determined by the appended claims and their legal equivalents, rather than by the examples given. All references cited herein are incorporated herein in their entirety by reference.

WHAT IS CLAIMED IS:

1. A method of producing polysaccharides which are non glycogen like in a host comprising:

a. transforming a host capable of being used in a fermentation process, with genes selected from the group which produce starch synthesizing enzymes, glycogen synthesizing enzymes such that the host produces nonglycogen like starch, and

b. employing the host in a fermentation process wherein the fermentation process produces polysaccharides.

2. A method according to claim 1 wherein the host is bacteria.

3. A host transformed to carry a gene active in glycogen production, and at least one nonstarch branching gene active in the production of at least one of the following polysaccharides amylopectin and amylose in it original host.

4. A host according to claim 3 wherein the host is a monocot.

5. A host according to claim 3 wherein the host is a dicot.

6. A host according to claim 3 wherein the host is a plant.

7. A host according to claim 3 wherein the host is a bacteria.

8. A host according to claim 3 wherein the host is a cereal bearing plant.

9. A host according to claim 3 wherein the bacterial gene is selected from the group consisting of glgC gene, glgA gene, glgB gene.

10. A host according to claim 3 wherein at least one nonstarch branching genes active in the production of at least one of the following polysaccharides amylopectin and amylose in

it original plant is selected from the group consisting of starch soluble starch synthase I, II, III genes and debranching enzyme gene (su1), GBSS gene, sh2 gene and bt2 gene.

11. A host according to claim 3 including at least one of the starch branching enzyme genes.

12. A host according to claim 11 including the starch branching enzyme gene BEI gene.

13. A host according to claim 11 including the starch branching enzyme gene BEII gene.

14. A host according to claim 12 including the starch branching enzyme gene BEII gene.

15. A host transformed to carry a gene active in ADPG production, and at least one starch gene active in the production of at least one of the following polysaccharides amylopectin and amylose in it original host wherein the host produces polysaccharides that are plant like starch and not glycogen like.

16. A host transformed to carry a pyrophosphatase gene, glycogen synthase gene,

17. A host according to claim 1 wherein the gene active in glycogen production is a bacterial gene.

18. A host deficient in alpha 1,4 glucan synthesizing ability and alpha 1,4-1,6 branching enzyme capability transformed to express at least one a plant starch soluble synthesis gene.

19. A host according to claim 18 including being transformed to express at least one gene encoding for debranching enzyme.

20. A host according to claim 18wherein said gene is encoding for starch soluble synthase enzyme I.

21. A host according to claim 18wherein said gene is encoding for starch soluble synthase enzyme II.

22. A host according to claim 18wherein said gene is encoding for starch soluble synthase enzyme III.

23. A host according to claim 18 including being transformed to express at least one gene encoding for starch branching enzyme.

24. A host according to claim 23 wherein the starch branching enzyme is BEI.

25. A host according to claim 23 wherein the starch branching enzyme is BEII.

26. A plasmid wherein said plasmid is in a carrier host and said plasmid contains the SSII gene with the n terminus GENVMNVIVV wherein the gene is approximately 1561 base pairs in length.

27. A Plasmid according to claim on e wherein said host is a bacterial host.

28. A host according to claim two wherein the host is a wild type E. Coli.

29. A new`polysaccharide produced by a transformed host comprising:
said host having a wildtype, said wildtype of said host does not produce said new polysaccharide, said transformed host expressing at least two exogenous starch synthesis genes, said genes are selected from a group consisting of soluble starch synthesis genes such as SSI SSII SSIII, wherein the transformed host is capable of producing such new polysaccharide.

30. The new polysaccharide of claim 29 wherein said host also expresses the exogenous genes selected from the following group consisting of bacterial glycogen inducing genes.

31. The new polysaccharide of claim 29 wherein said host also expresses the exogenous genes selected from the following group consisting of plant granule bound enzymes.

32. A method according to claim one wherein the host is fungal.

33. A method according to claim 32 wherein the host is yeast.

34. A method according to claim one wherein said glycogen synthesizing genes include glgC, glgA, glgB genes.

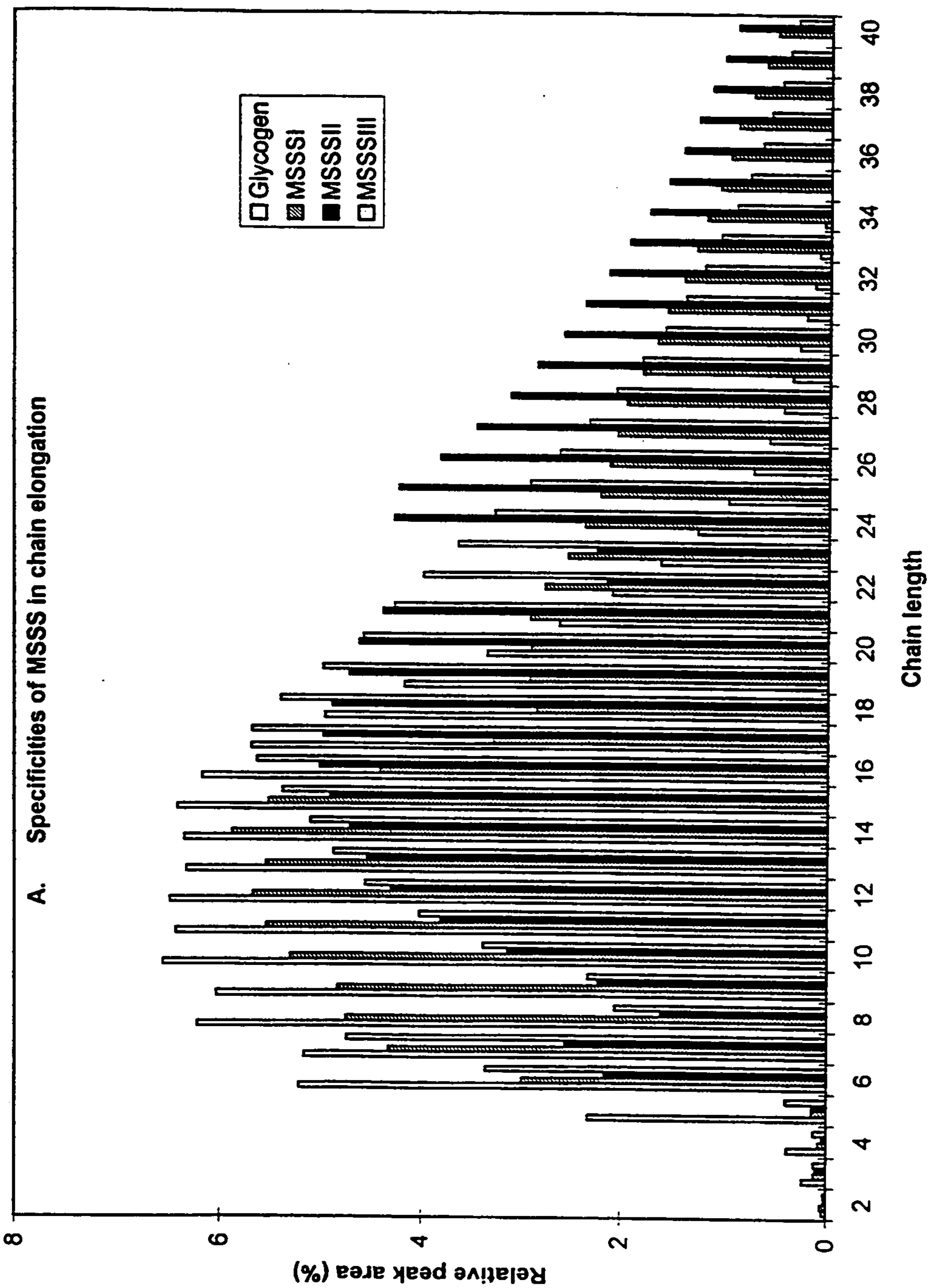
35. A method according to claim one wherein said genes which produce starch synthesizing enzymes include genes encoding for starch soluble synthases I, II, III.

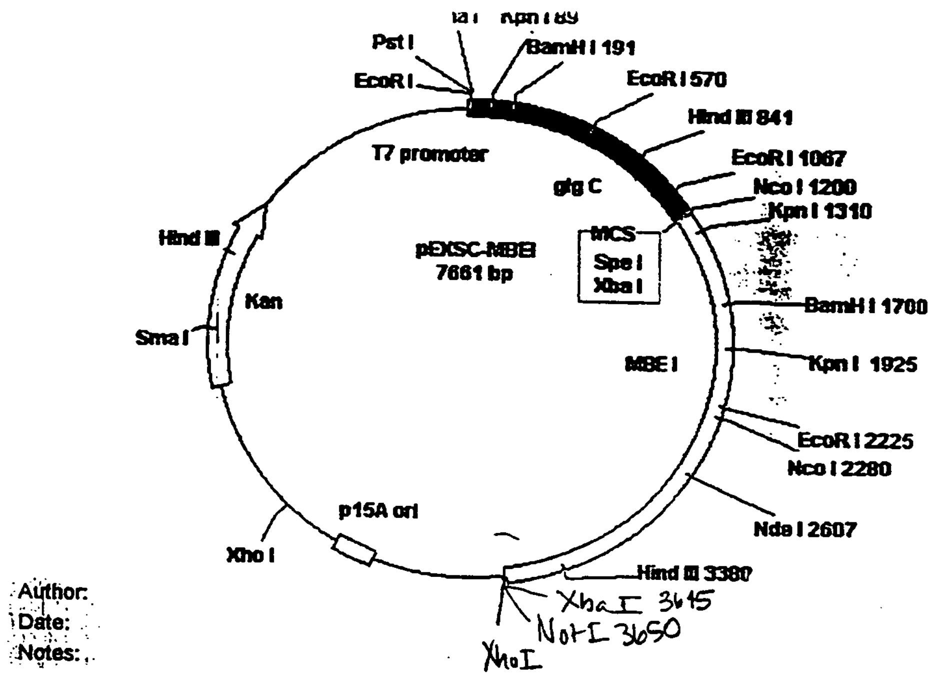
36. A method according to claim one wherein said genes which produce starch synthesizing enzymes include genes encoding for and debranching enzyme and branching enzymes.

37. The new polysaccharide of claim 30 wherein said bacterial glycogen inducing genes are selected from the group consisting of glgC, glgA, glgB.

38. The new polysaccharide of claim 29 wherein said starch synthesis genes are selected from the group consisting of BEI and BEII.

Fig 1

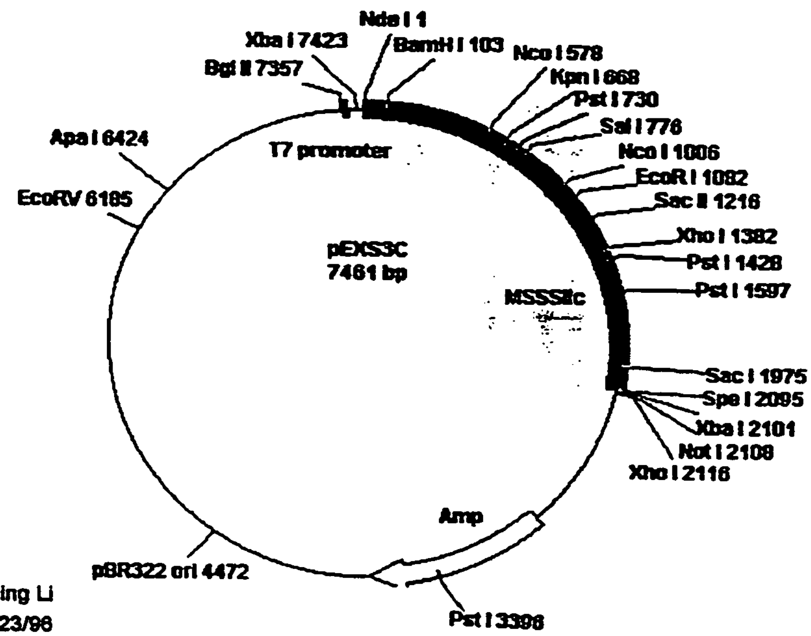

A. Specificities of MSSS in chain elongation
Glycogen
MSSSI
MSSSII
MSSSIII
Relative peak area (%)
Chain length
0
2
4
6
8
2
4
6
8
10
12
14
16
18
20
22
24
26
28
30
32
34
36
38
40



Pst I
Kpn I 89
BamH I 191
EcoR I
EcoR I 570
Hind III 841
EcoR I 1067
Nco I 1200
Kpn I 1310
T7 promoter
glg C
MCS
Spe I
Xba I
pEXSC-MBEI
7661 bp
Hind III
Kan
Sma I
BamH I 1700
Kpn I 1925
MBE I
EcoR I 2225
Nco I 2280
Nde I 2607
p15A ori
Xho I
Hind III 3380
Xba I 3645
Not I 3650
Xho I
Author:
Date:
Notes:

Fig. 2

Fig 3



Author: Ping Li
Date: 7/23/96
Notes:

pEXS3C is the 1082 bp Nde I-EcoR I fragment containing the N-terminus of MSSSIIc (from MSSSIIc in pBSK) subcloned into the Nde I-EcoR I sites of pEXS3a, replacing the N-terminus of IIa with the longer IIc N-terminus. MSSSIIc is the mature maize STS2 and is 2090 bp long. The following sites are NOT contained in the MSSSIIc insert: Apa I, Bgl II, EcoR V, Not I, Spe I, and Xba I.

see Ping's Notebook #1 pp. 149, 159, 160.

vector: pET-21a

N-terminus of MSSSIIc: AEAEAGGKD

Fig 4

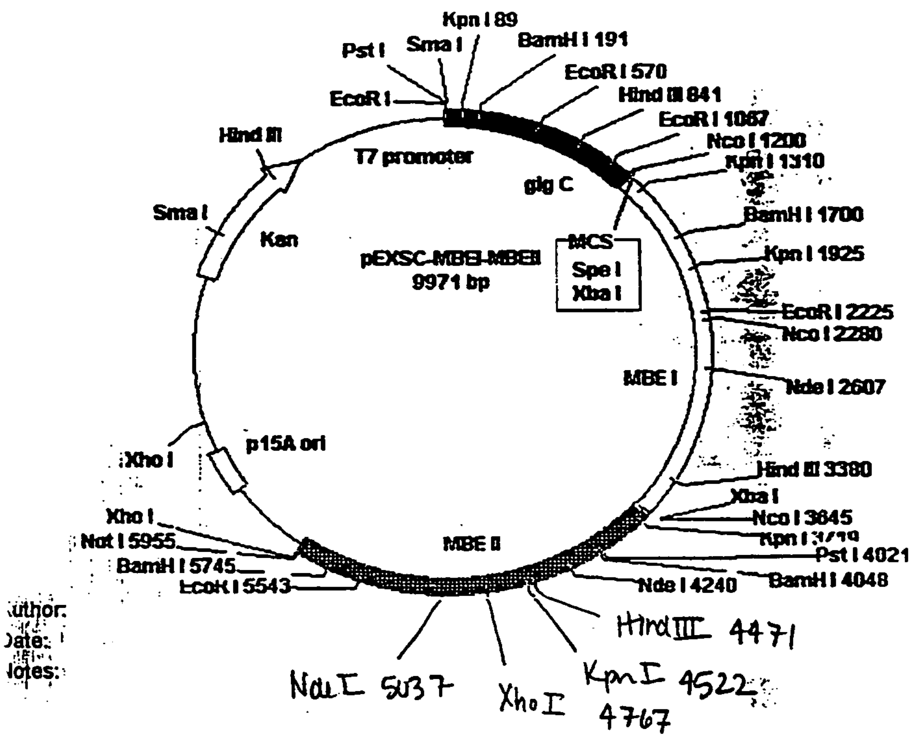
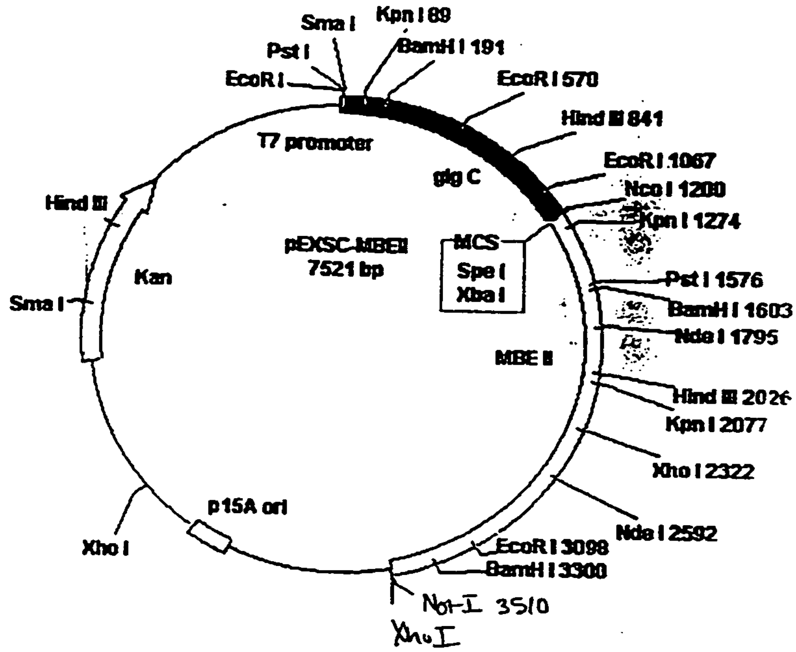
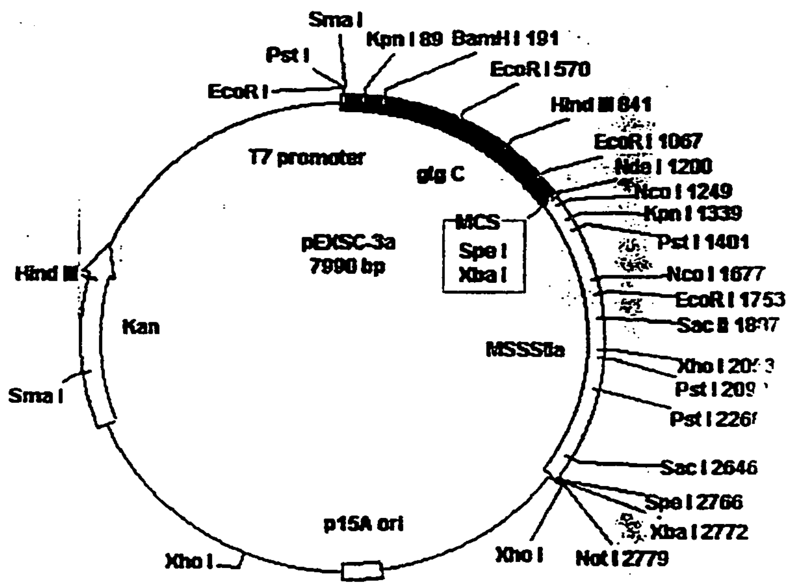

Kpn I 89
Pst I
Sma I
BamH I 191
EcoR I 570
EcoR I
Hind III 841
EcoR I 1067
Nco I 1200
Kpn I 1310
Hind III
T7 promoter
glg C
Sma I
Kan
BamH I 1700
pEXSC-MBEI-MBEII
9971 bp
MCS
Spe I
Xba I
Kpn I 1925
EcoR I 2225
Nco I 2280
MBE I
Nde I 2607
Xho I
p15A ori
Hind III 3380
Xba I
Xho I
Nco I 3645
Not I 5955
MBE II
Pst I 4021
BamH I 5745
BamH I 4048
EcoR I 5543
Nde I 4240
HindIII 4471
NdeI 5037
KpnI 4522
XhoI 4767
Author:
Date:
Notes:

Fig 5



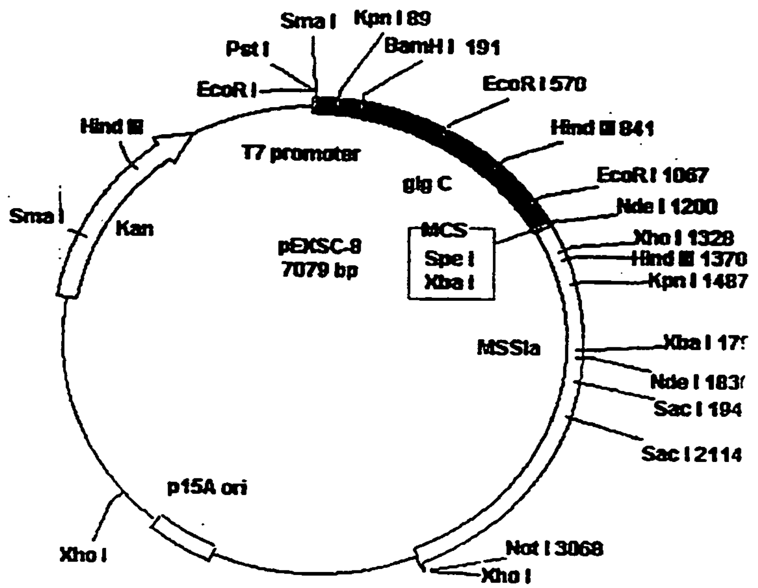
Author:
Date:
Notes:

Fig 6



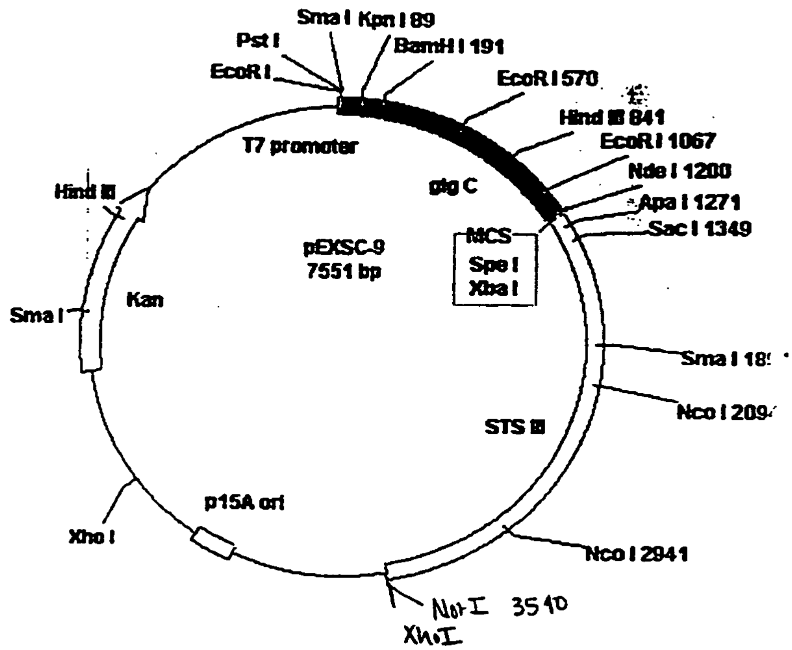
Author:
Date:
Notes:

Fig 7



uthor:
Date:
Notes:

Fig 8



Author:
Date:
Notes:

Fig.9

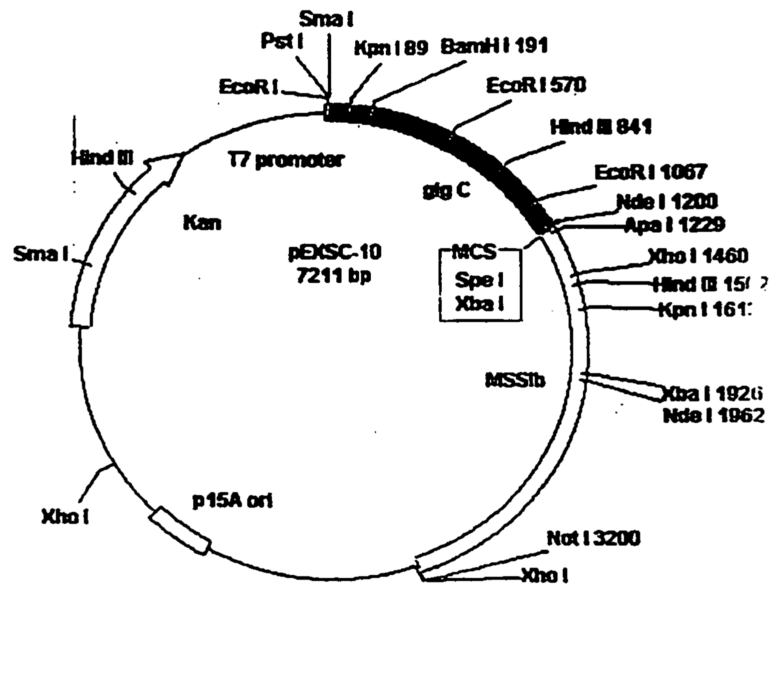
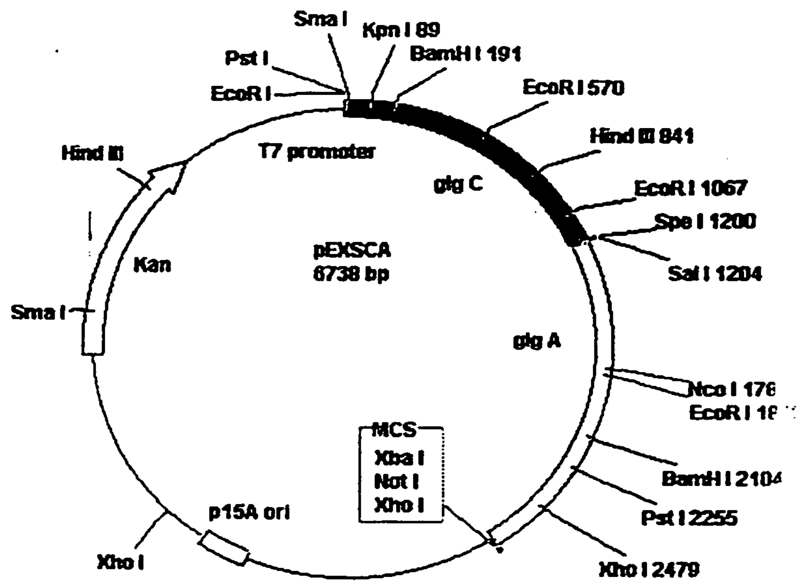

Sma I
Pst I
Kpn I 89
BamH I 191
EcoR I
EcoR I 570
Hind III 841
Hind III
T7 promoter
EcoR I 1067
gig C
Nde I 1200
Apa I 1229
Kan
Sma I
pEXSC-10
7211 bp
MCS
Spe I
Xba I
Xho I 1460
Kpn I
MSSIb
Xba I
Nde I 1962
Xho I
p15A ori
Not I 3200
Xho I
uthor:
Date:
Notes:

Fig. 10



uthor:
Date:
Notes:

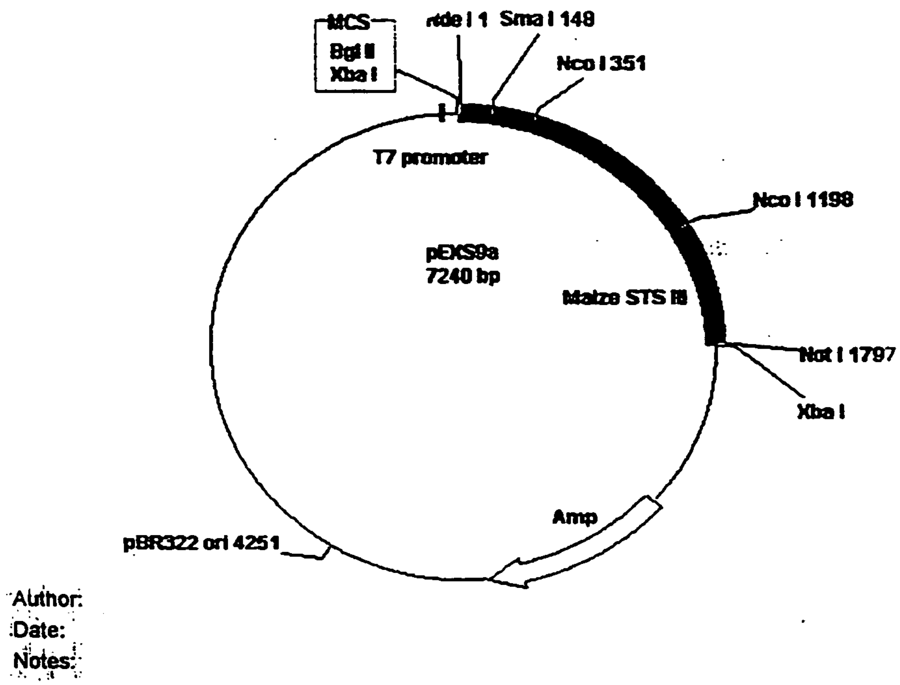
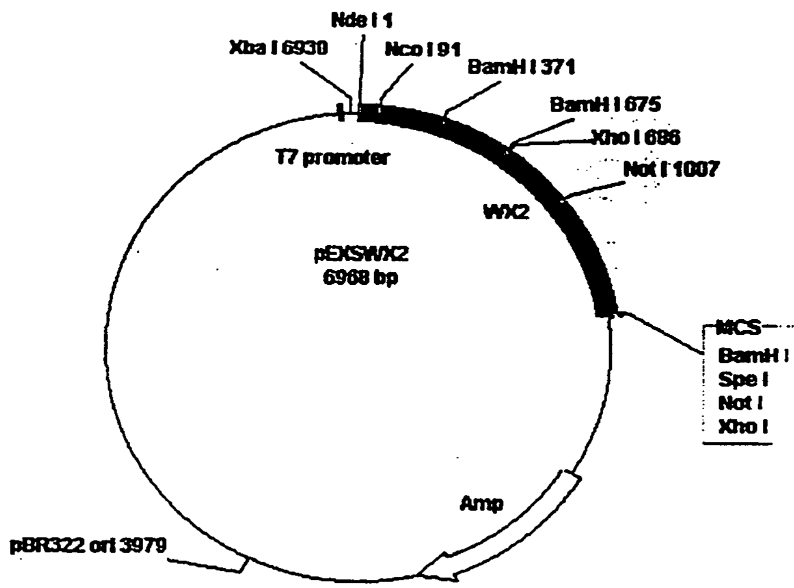

MCS
Bgl II
Xba I
Nde I 1
Sma I 149
Nco I 351
T7 promoter
Nco I 1198
pEXS9a
7240 bp
Maize STS III
Not I 1797
Xba I
Amp
pBR322 ori 4251
Author:
Date:
Notes:

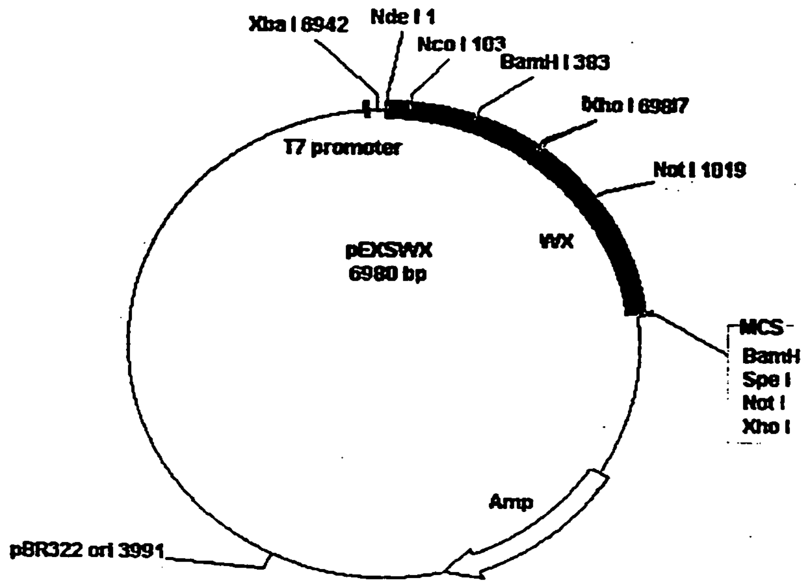
Fig. 11

Fig 12



Author:
Date:
Notes:

Fig 13



uthor:
Date:
lotes:

Fig. 14

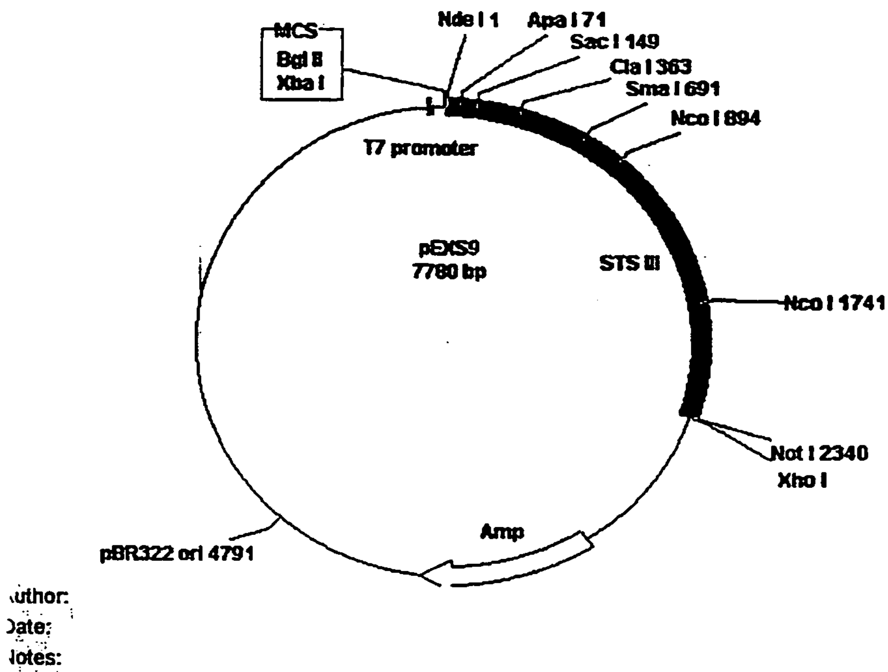
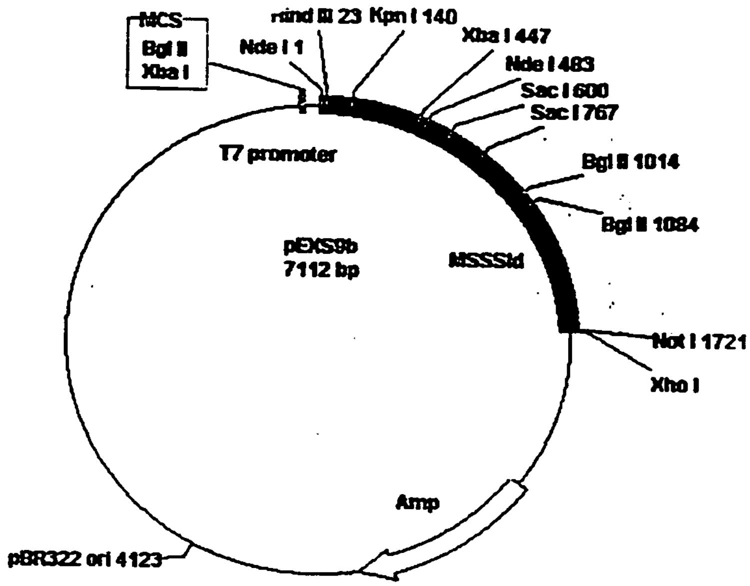
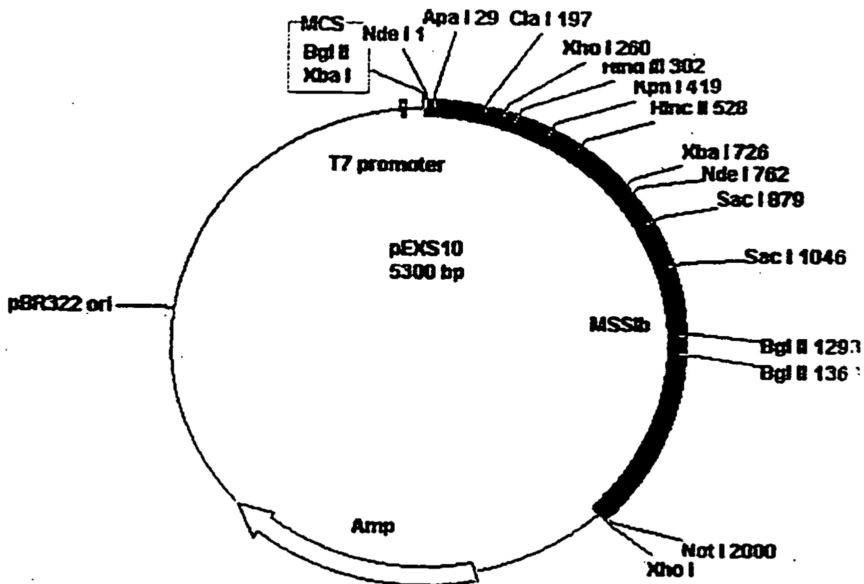

MCS
Bgl II
Xba I
Nde I 1
Apa I 71
Sac I 149
Cla I 363
Sma I 691
Nco I 894
T7 promoter
pEXS9
7780 bp
STS III
Nco I 1741
Not I 2340
Xho I
Amp
pBR322 ori 4791

Fig. 15



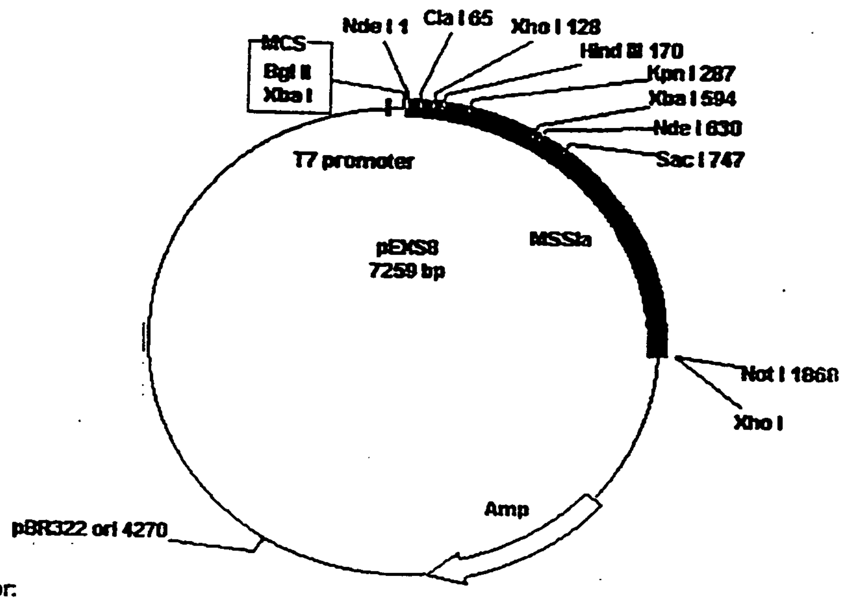
Author:
Date:
Notes:

Fig. 16



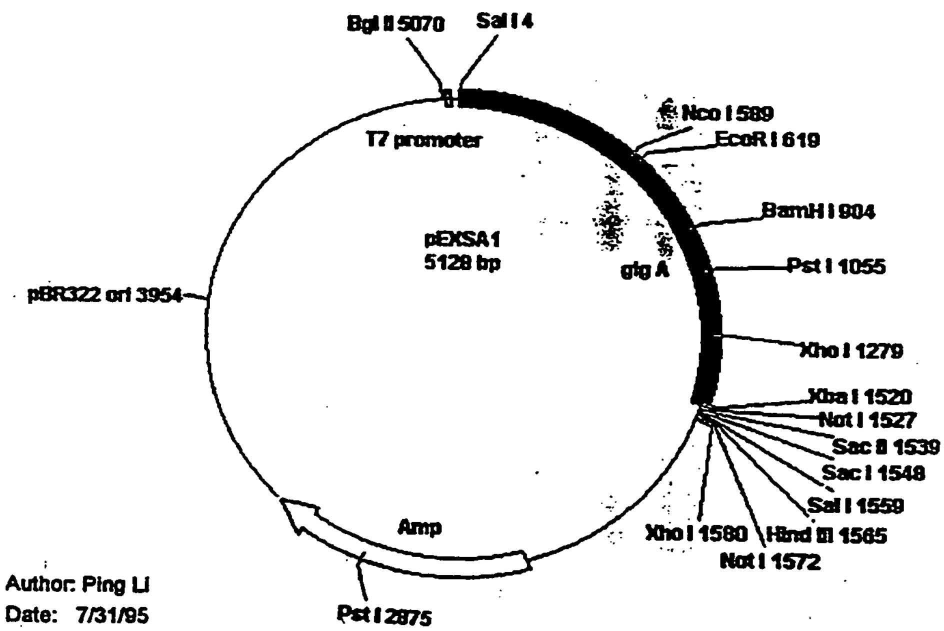
Author:
Date:
Notes:

Fig 17



Author:
Date:
Notes:

Fig 18



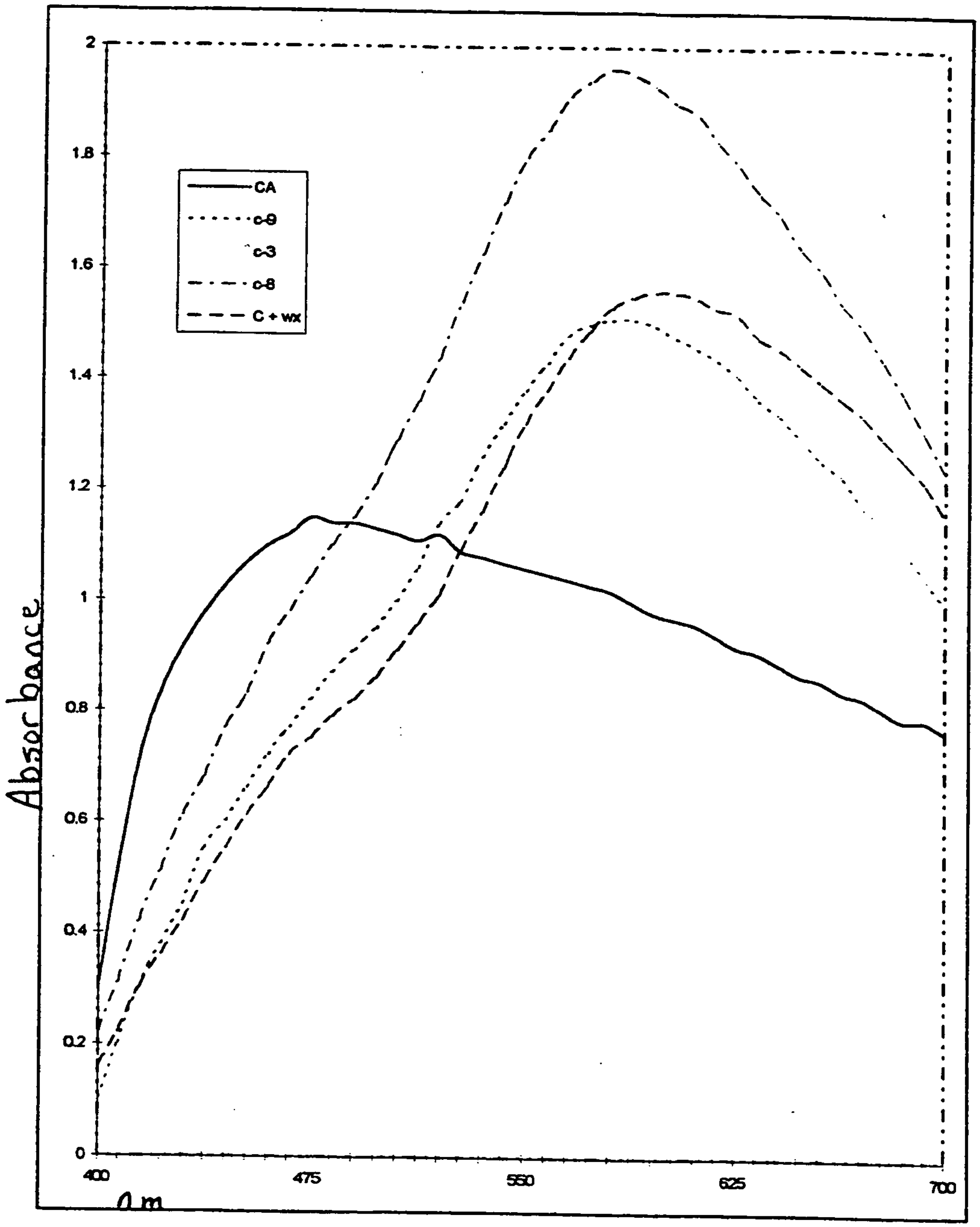
Author: Ping Li
Date: 7/31/95
Notes:

pEXSA1 is a 1551 bp Spe I-Sac I fragment containing glg A (from glgA in pBSK) subcloned into the Xba I-Sac I sites of pET-23d.

see "glg A,B,C" notebook p. 71.

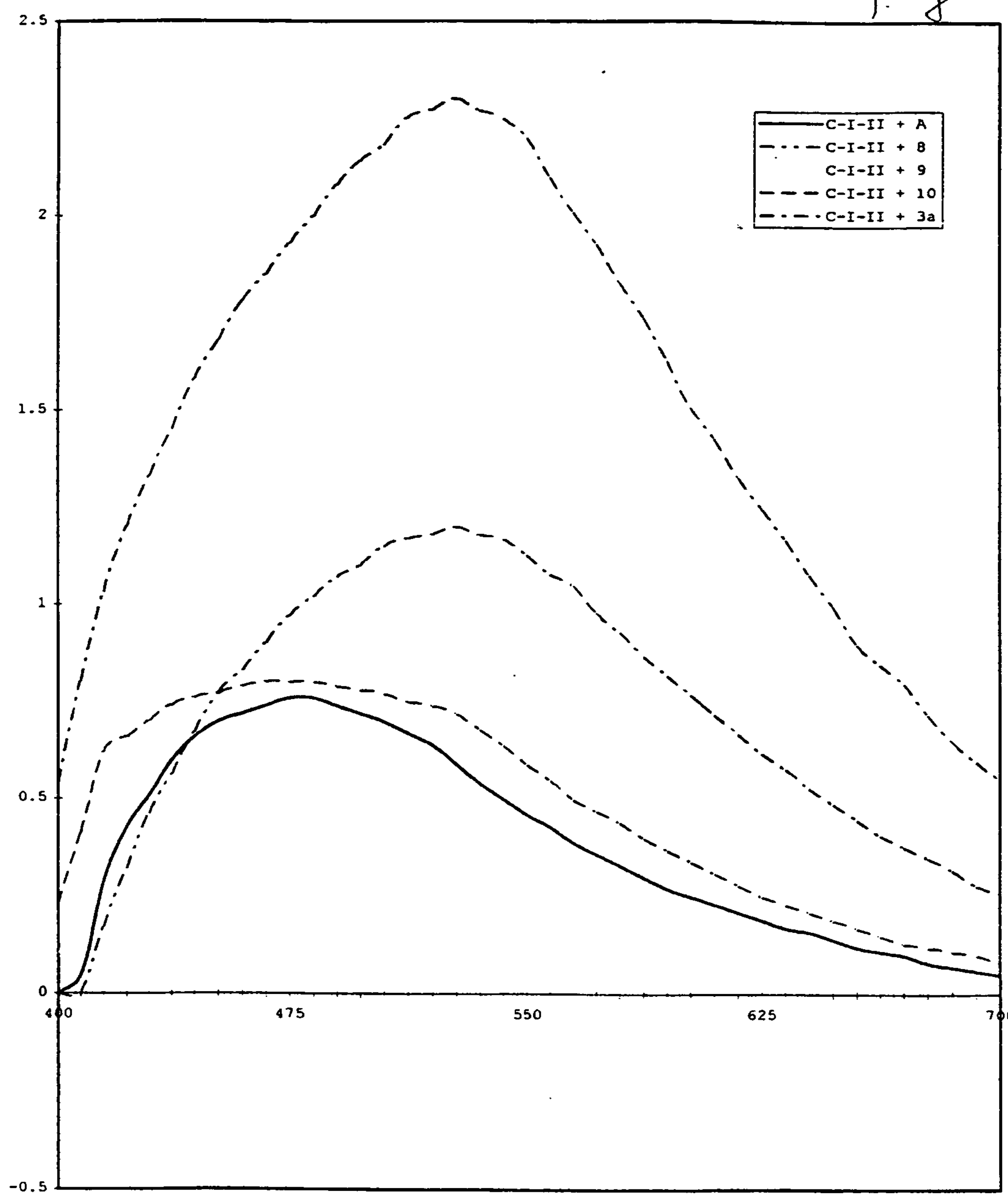
Sheet1

Fig 19



Sheet1 Chart 1

Fig 20



Page 1

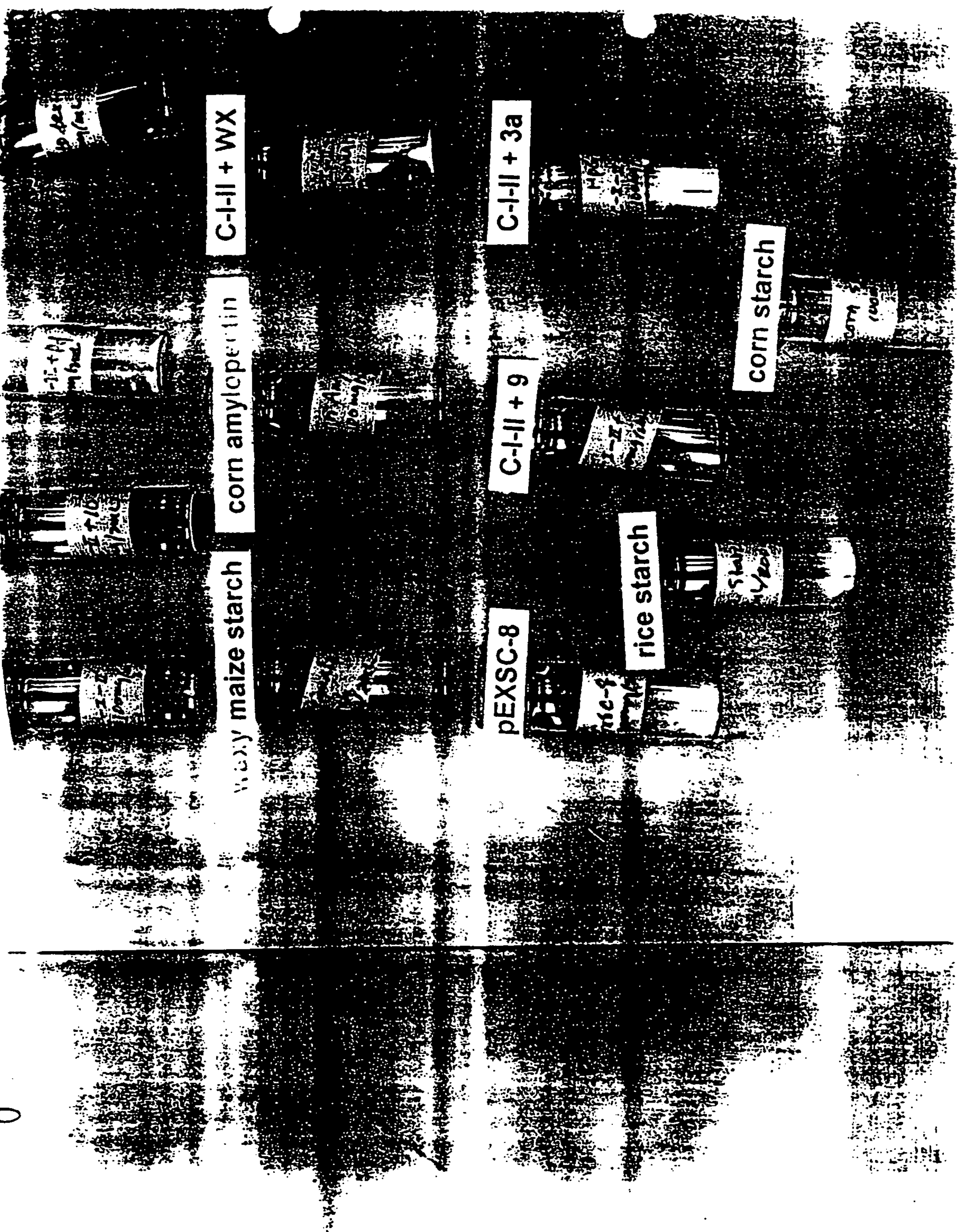
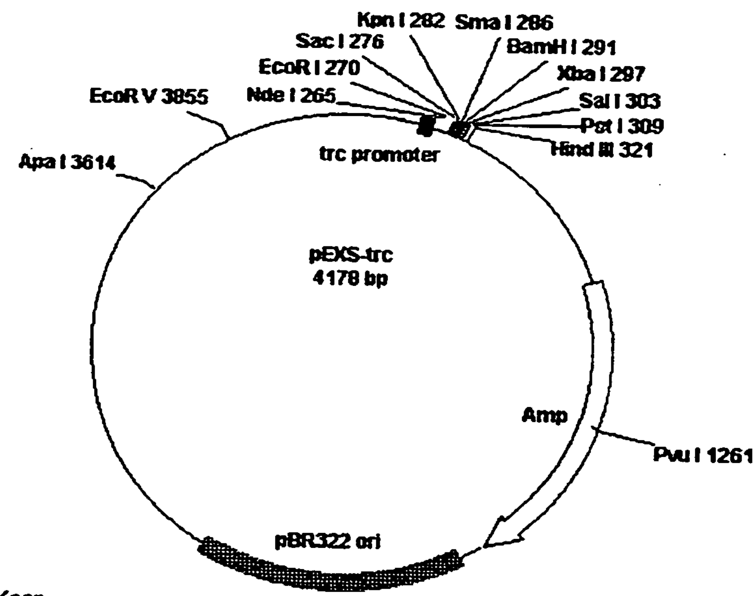

C-I-II + WX
corn amylopectin
waxy maize starch
C-I-II + 3a
C-I-II + 9
pEXSC-8
corn starch
rice starch

Fig 21

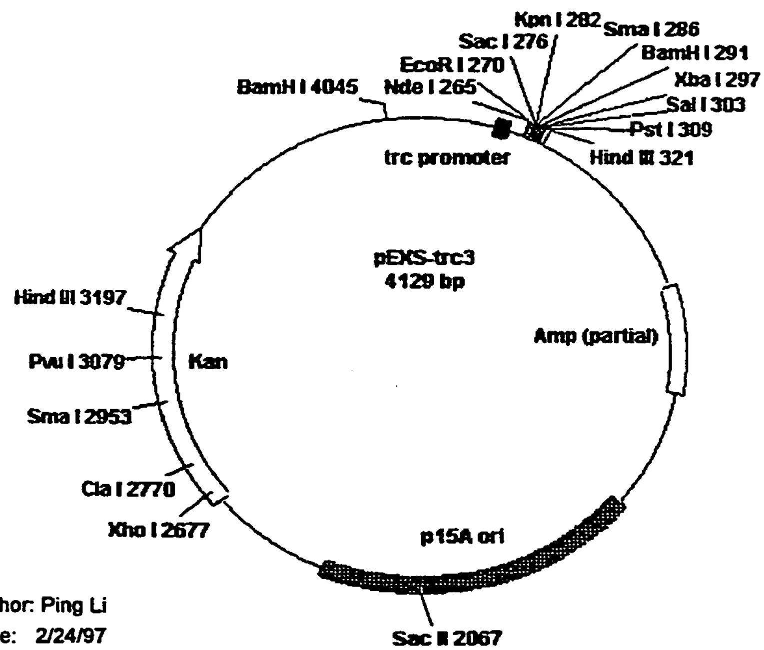
Fig 22



Author: McKean
Date: 2/10/97
Notes:

pEXS-trc is pTrc99A-NdeI which has been mutagenized. (Nco I site in multiple cloning site of pTrc99A-NdeI is mutagenized to Nde I using primers EXS63 and EXS64.) pEXS-trc contains only one Nde I site and no Nco I sites. The following sites are NOT contained in pEXS-trc: Bgl II, Cla I, Nco I, Not I, Sac II, SnaB I, Spe I, and Xho I.
see Angela's Notebook #1 pp. 231, 242-244, 246, 248-250.

Fig 23



Author: Ping Li
Date: 2/24/97
Notes:

pEXS-trc3 is pEXS-trc1 cut with Bgl I (filled in)-Sca I and religated, deleting most of the Amp gene (304 nt from the 5' end remain). The following sites are NOT contained in pEXS-trc3: Apa I, Bgl II, EcoR V, Nco I, Not I, SnaB I, and Spe I.
see Ping's Notebook #2 pp. 204, 209.

Fig. 24

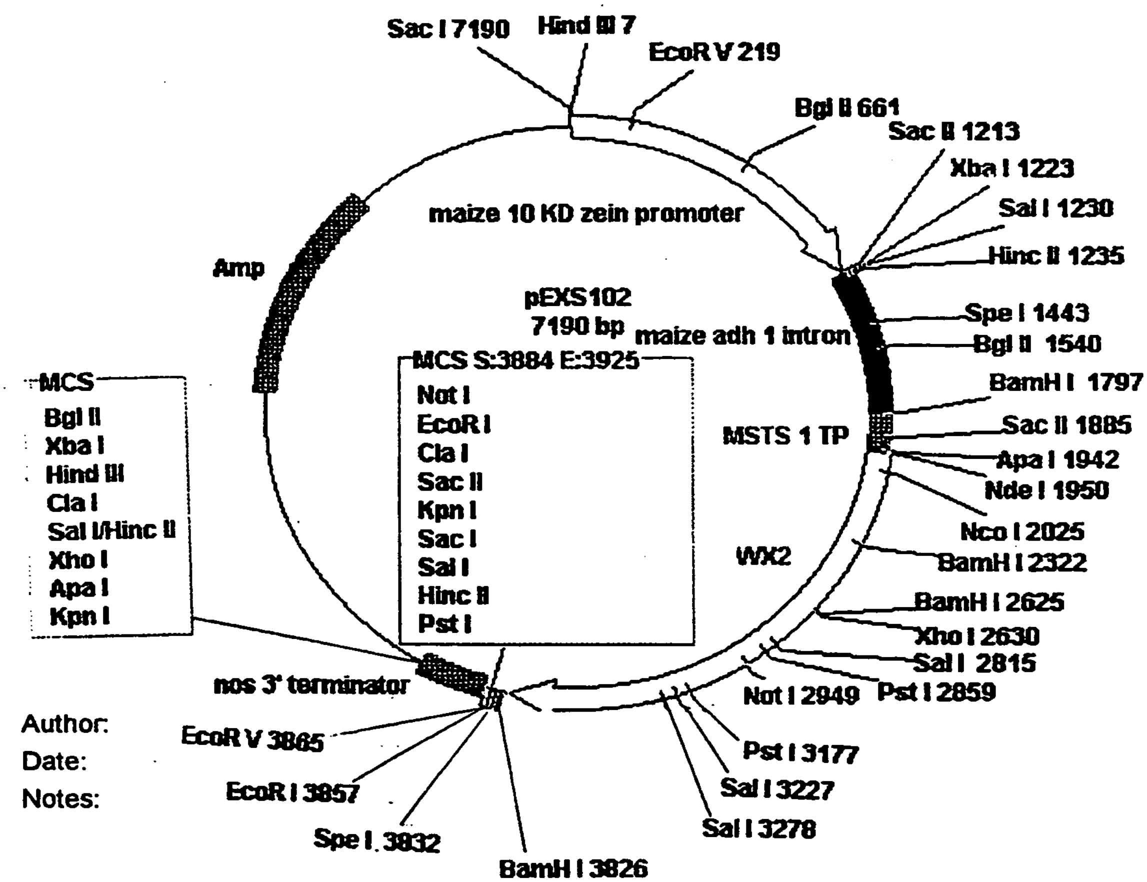

Sac I 7190
Hind III 7
EcoR V 219
Bgl II 661
Sac II 1213
Xba I 1223
Sal I 1230
Hinc II 1235
Spe I 1443
Bgl II 1540
BamH I 1797
Sac II 1885
Apa I 1942
Nde I 1950
Nco I 2025
BamH I 2322
BamH I 2625
Xho I 2630
Sal I 2815
Pst I 2859
Not I 2949
Pst I 3177
Sal I 3227
Sal I 3278
BamH I 3826
Spe I 3832
EcoR I 3857
EcoR V 3865
nos 3' terminator
maize 10 KD zein promoter
Amp
pEXS102
7190 bp
maize adh 1 intron
MSTS 1 TP
WX2
MCS S:3884 E:3925
Not I
EcoR I
Cla I
Sac II
Kpn I
Sac I
Sal I
Hinc II
Pst I
MCS
Bgl II
Xba I
Hind III
Cla I
Sal I/Hinc II
Xho I
Apa I
Kpn I
Author:
Date:
Notes:

Fig. 25

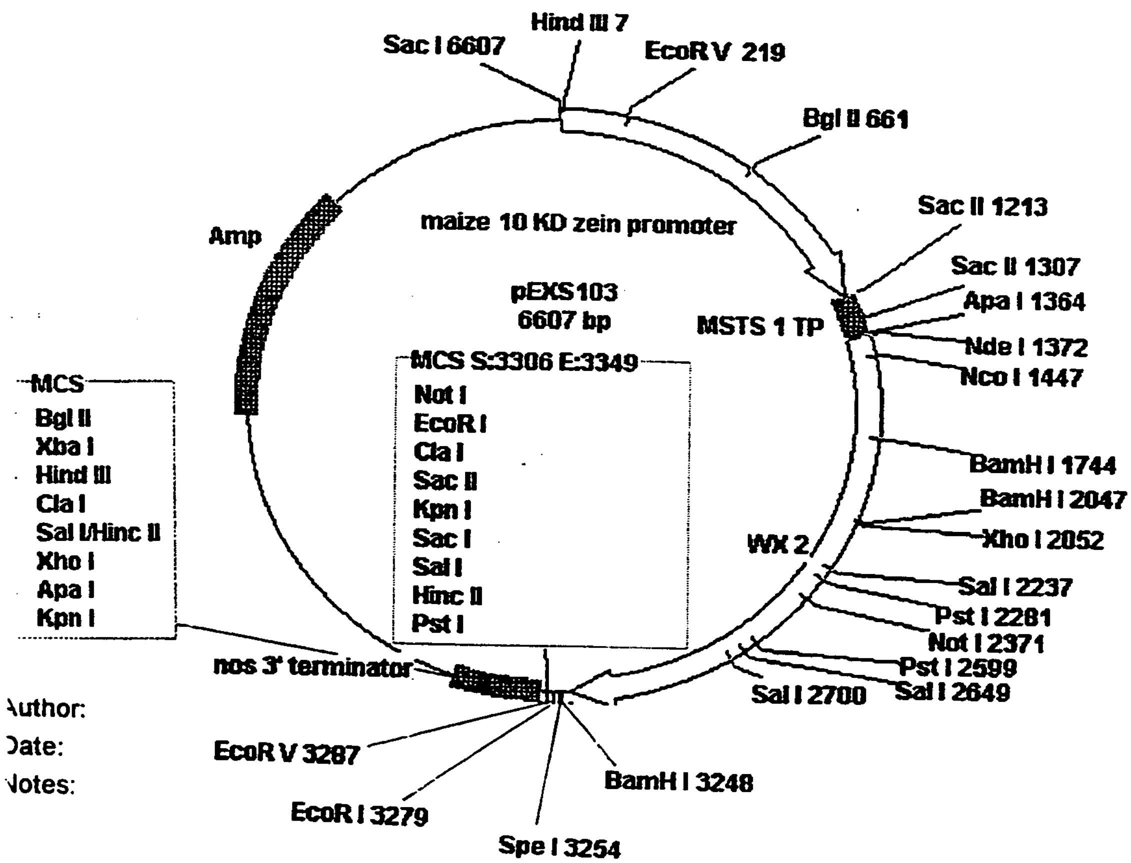

Hind III 7
Sac I 6607
EcoR V 219
Bgl II 661
Sac II 1213
Sac II 1307
Apa I 1364
Nde I 1372
Nco I 1447
BamH I 1744
BamH I 2047
Xho I 2052
Sal I 2237
Pst I 2281
Not I 2371
Pst I 2599
Sal I 2649
Sal I 2700
BamH I 3248
Spe I 3254
EcoR I 3279
EcoR V 3287
maize 10 KD zein promoter
Amp
pEXS103
6607 bp
MSTS 1 TP
WX 2
nos 3' terminator
MCS S:3306 E:3349
Not I
EcoR I
Cla I
Sac II
Kpn I
Sac I
Sal I
Hinc II
Pst I
MCS
Bgl II
Xba I
Hind III
Cla I
Sal I/Hinc II
Xho I
Apa I
Kpn I
Author:
Date:
Notes:

Fig. 26

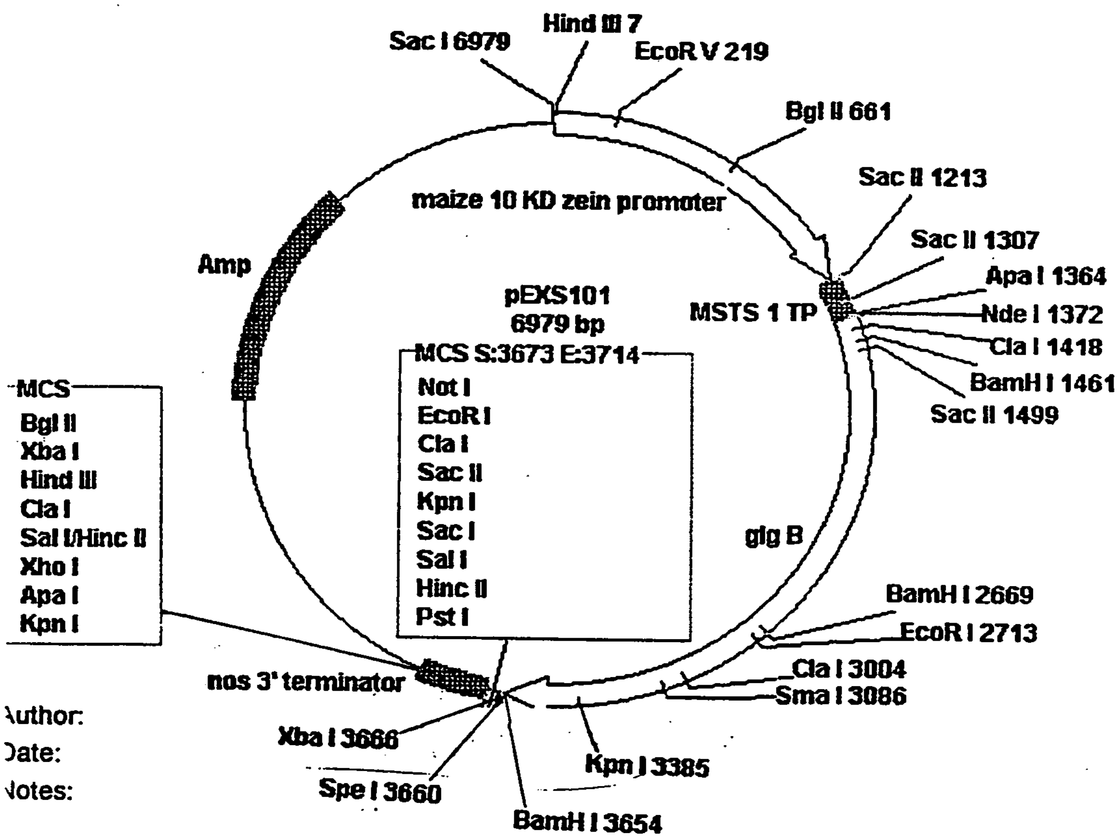

Sac I 6979
Hind III 7
EcoR V 219
Bgl II 661
Sac II 1213
Sac II 1307
Apa I 1364
Nde I 1372
Cla I 1418
BamH I 1461
Sac II 1499
maize 10 KD zein promoter
MSTS 1 TP
Amp
pEXS101
6979 bp
MCS S:3673 E:3714
Not I
EcoR I
Cla I
Sac II
Kpn I
Sac I
Sal I
Hinc II
Pst I
glg B
MCS
Bgl II
Xba I
Hind III
Cla I
Sal I/Hinc II
Xho I
Apa I
Kpn I
BamH I 2669
EcoR I 2713
Cla I 3004
Sma I 3086
nos 3' terminator
Xba I 3666
Spe I 3660
BamH I 3654
Kpn I 3385
Author:
Date:
Notes:

Fig 27

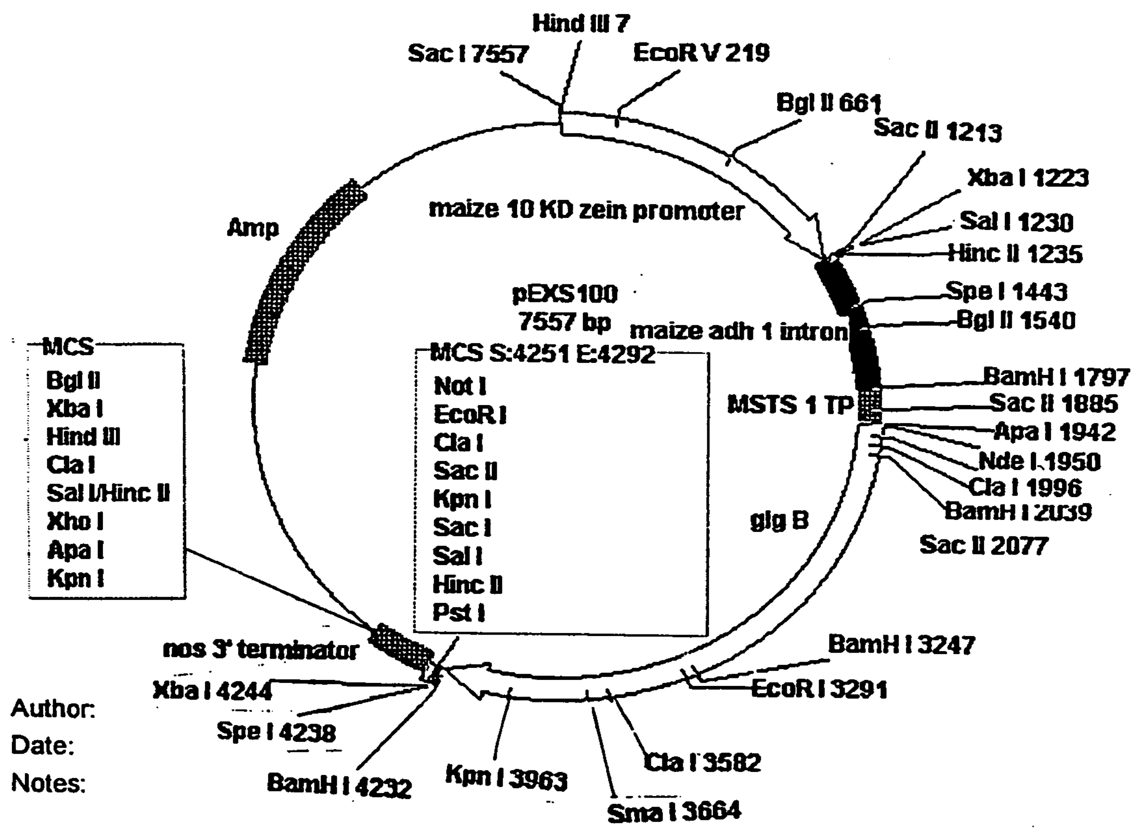
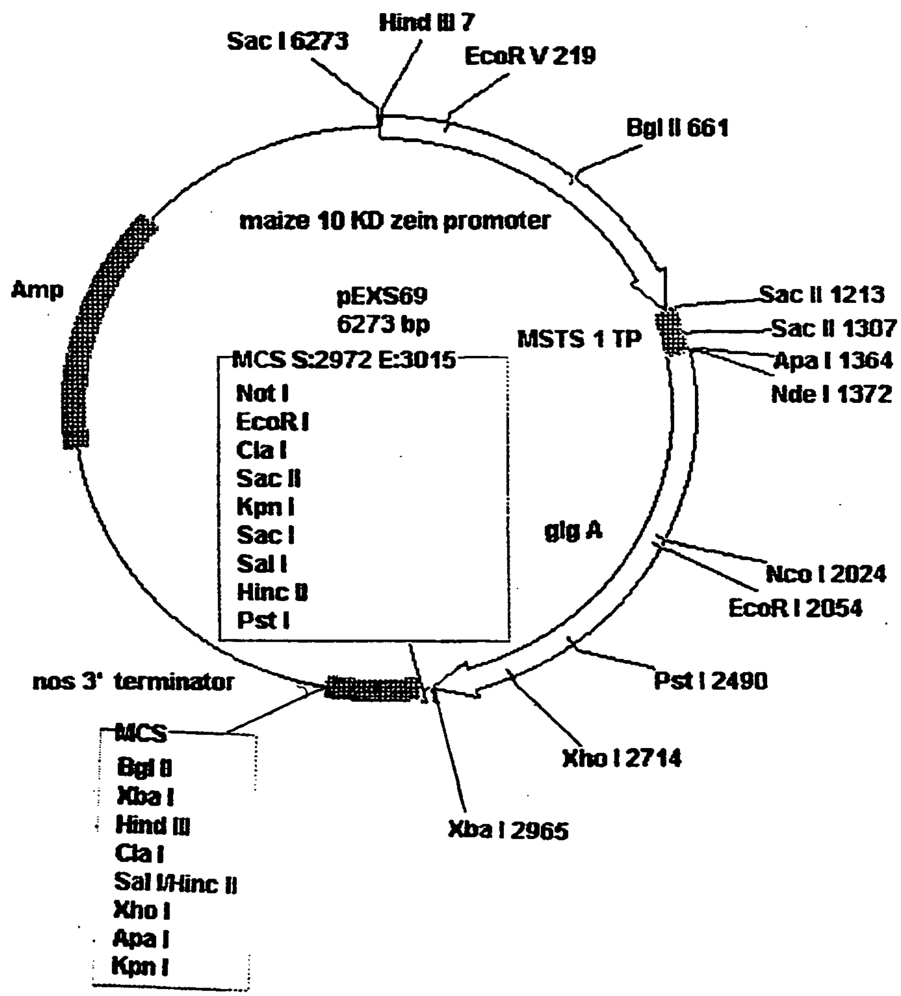

Hind III 7
Sac I 7557
EcoR V 219
Bgl II 661
Sac II 1213
Xba I 1223
Sal I 1230
Hinc II 1235
Spe I 1443
Bgl II 1540
BamH I 1797
Sac II 1885
Apa I 1942
Nde I 1950
Cla I 1996
BamH I 2039
Sac II 2077
BamH I 3247
EcoR I 3291
Cla I 3582
Sma I 3664
Kpn I 3963
BamH I 4232
Spe I 4238
Xba I 4244
maize 10 KD zein promoter
maize adh 1 intron
MSTS 1 TP
glg B
nos 3' terminator
Amp
pEXS100
7557 bp
MCS S:4251 E:4292
Not I
EcoR I
Cla I
Sac II
Kpn I
Sac I
Sal I
Hinc II
Pst I
MCS
Bgl II
Xba I
Hind III
Cla I
Sal I/Hinc II
Xho I
Apa I
Kpn I
Author:
Date:
Notes:

Fig 28



Author:
Date:
Notes:

Fig 29

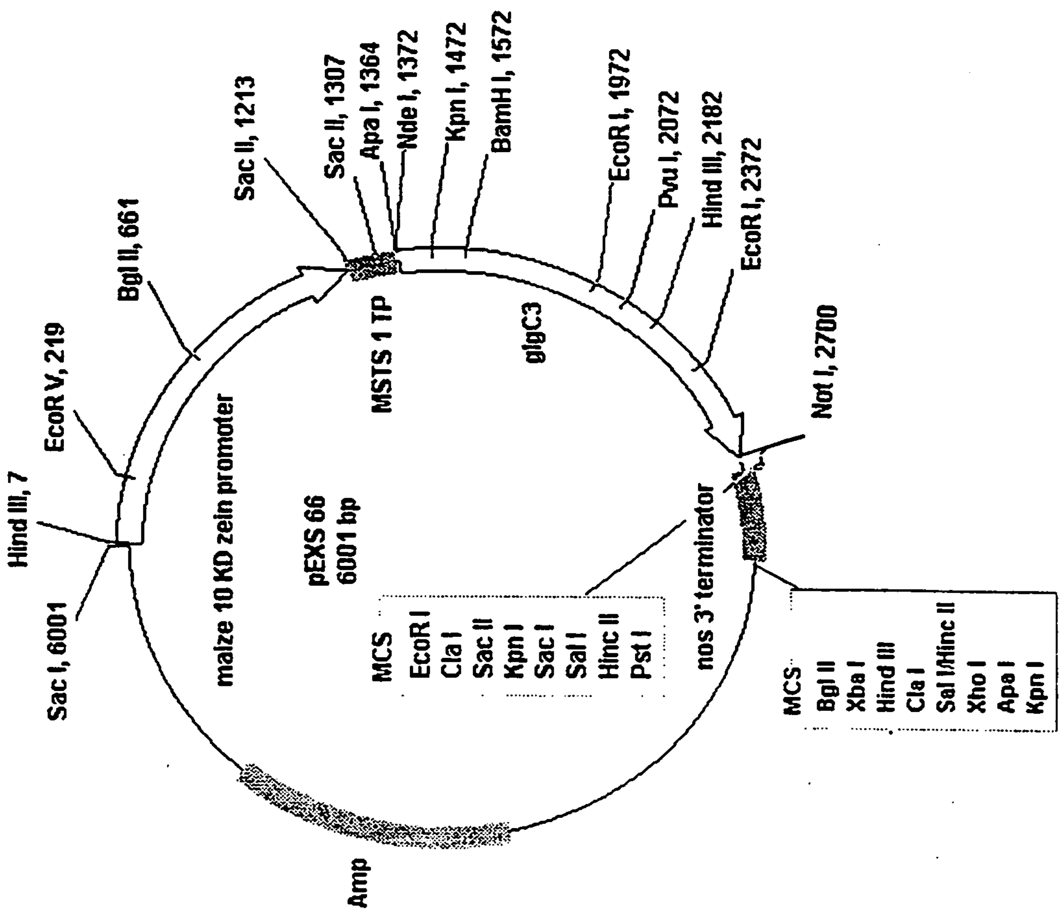
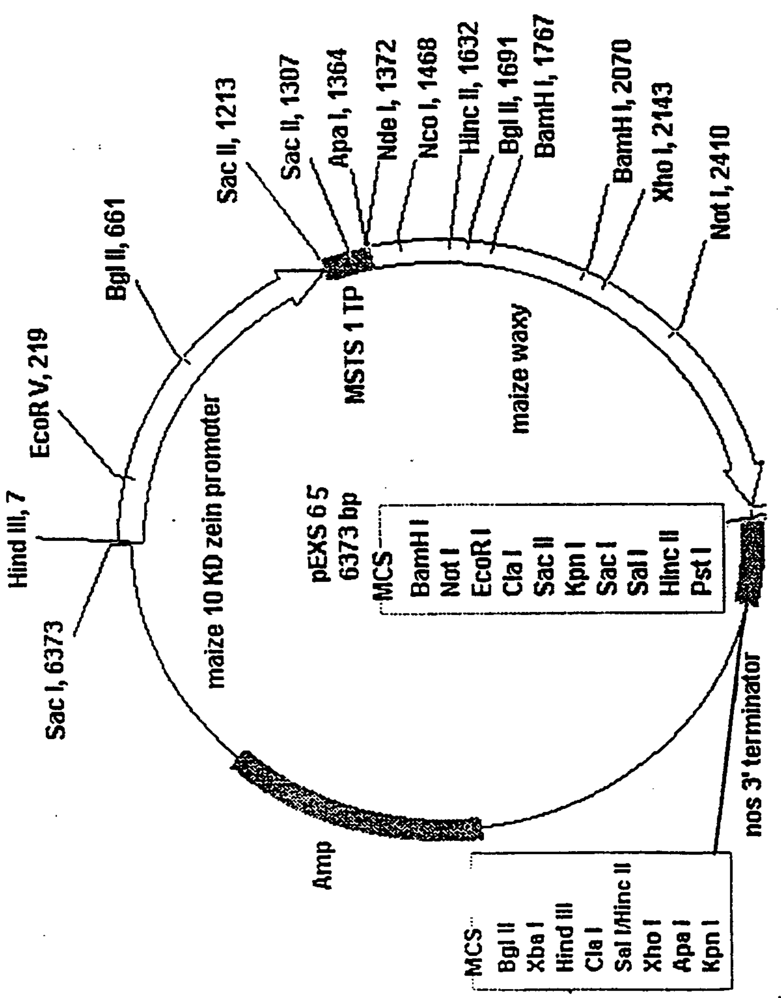

Hind III, 7
Sac I, 6001
EcoR V, 219
Bgl II, 661
Sac II, 1213
Sac II, 1307
Apa I, 1364
Nde I, 1372
Kpn I, 1472
BamH I, 1572
EcoR I, 1972
Pvu I, 2072
Hind III, 2182
EcoR I, 2372
Not I, 2700
maize 10 KD zein promoter
MSTS 1 TP
glgC3
pEXS 66
6001 bp
MCS
EcoR I
Cla I
Sac II
Kpn I
Sac I
Sal I
Hinc II
Pst I
nos 3' terminator
MCS
Bgl II
Xba I
Hind III
Cla I
Sal I/Hinc II
Xho I
Apa I
Kpn I
Amp

Fig 30



Waxy = maize Granule bound starch synthase

Fig 31

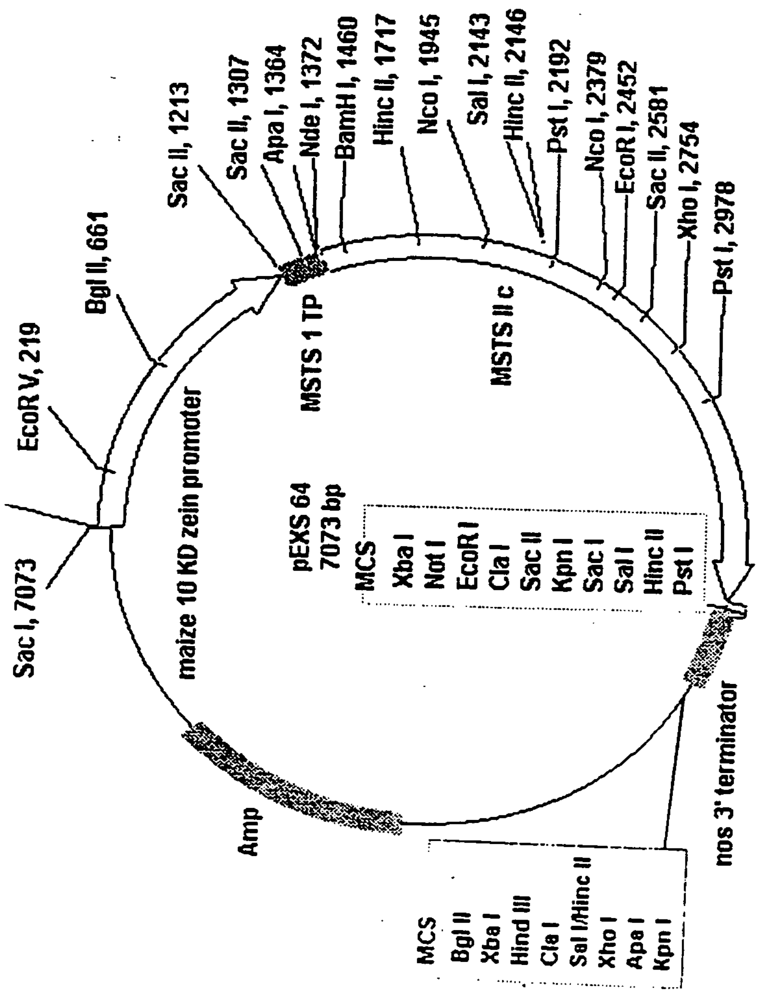

Sac I, 7073
EcoR V, 219
Bgl II, 661
Sac II, 1213
Sac II, 1307
Apa I, 1364
Nde I, 1372
BamH I, 1460
Hinc II, 1717
Nco I, 1945
Sal I, 2143
Hinc II, 2146
Pst I, 2192
Nco I, 2379
EcoR I, 2452
Sac II, 2581
Xho I, 2754
Pst I, 2978
MSTS 1 TP
MSTS II c
maize 10 KD zein promoter
pEXS 64
7073 bp
MCS
Xba I
Not I
EcoR I
Cla I
Sac II
Kpn I
Sac I
Sal I
Hinc II
Pst I
Amp
nos 3' terminator
MCS
Bgl II
Xba I
Hind III
Cla I
Sal I/Hinc II
Xho I
Apa I
Kpn I

Fig. 32

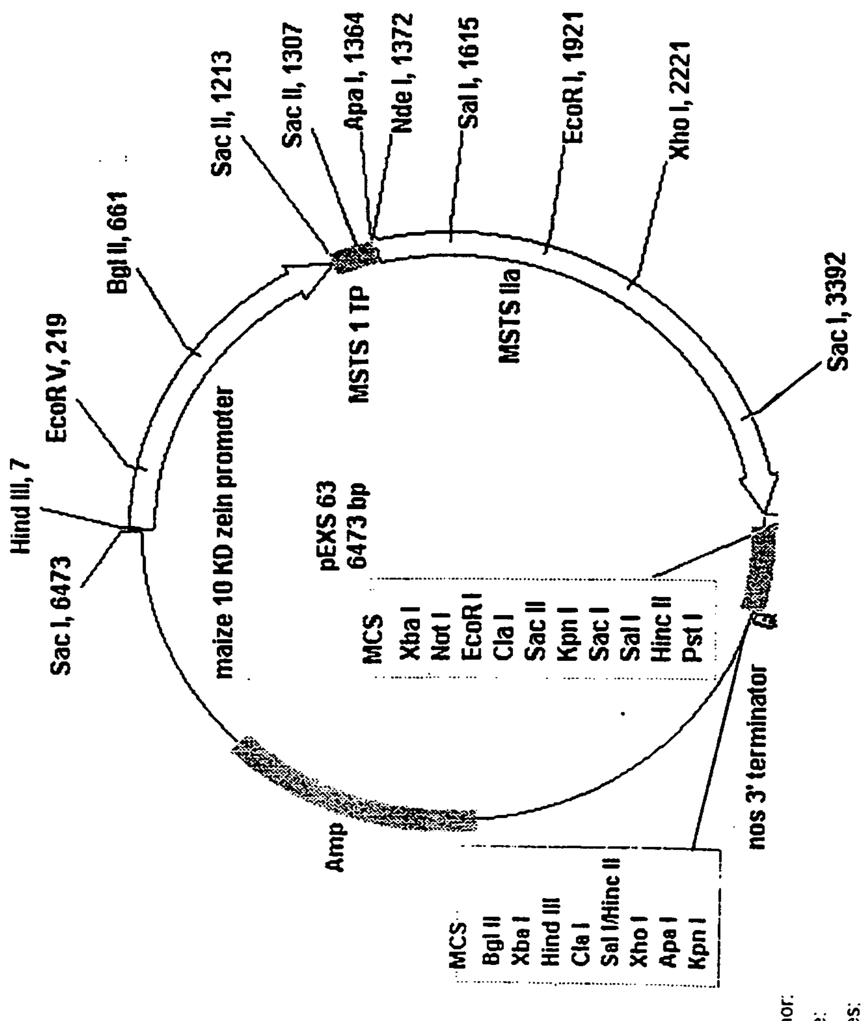
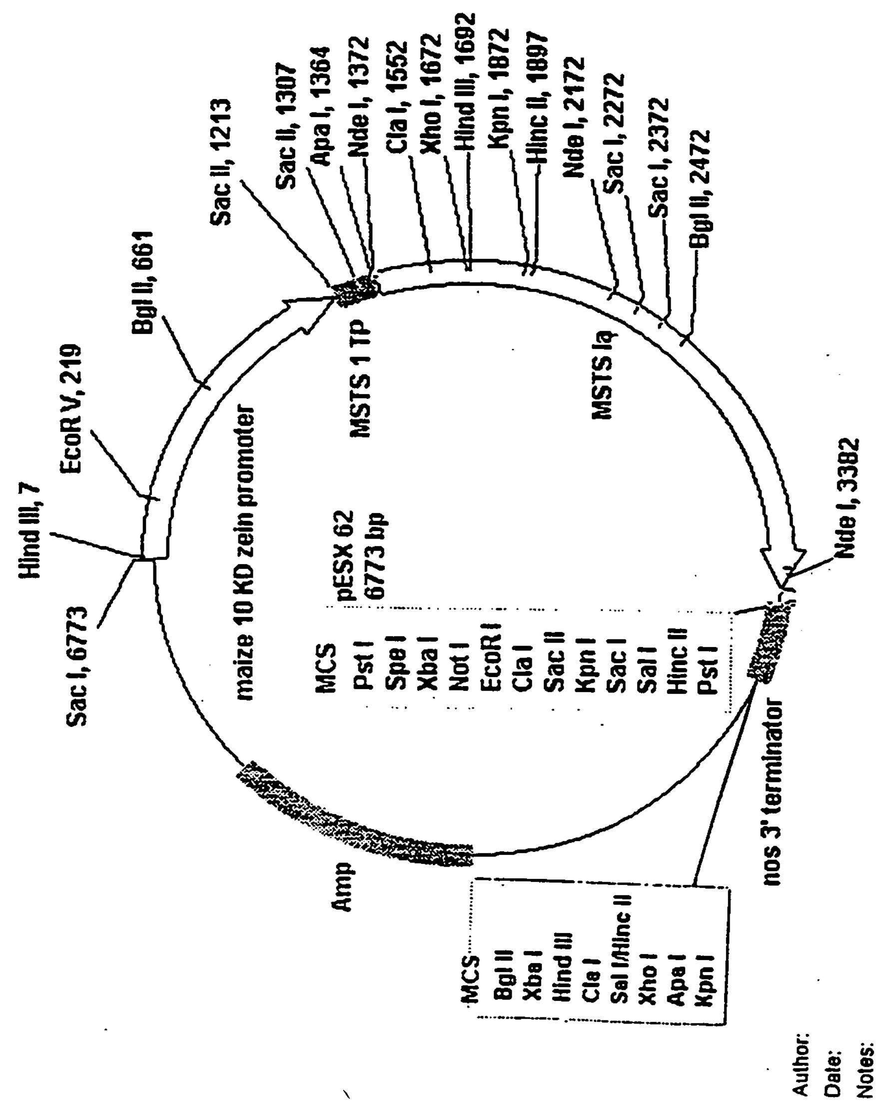

Sac I, 6473
Hind III, 7
EcoR V, 219
Bgl II, 661
Sac II, 1213
Sac II, 1307
Apa I, 1364
Nde I, 1372
Sal I, 1615
EcoR I, 1921
Xho I, 2221
Sac I, 3392
maize 10 KD zein promoter
MSTS 1 TP
MSTS IIa
pEXS 63
6473 bp
MCS
Xba I
Not I
EcoR I
Cla I
Sac II
Kpn I
Sac I
Sal I
Hinc II
Pst I
nos 3' terminator
Amp
MCS
Bgl II
Xba I
Hind III
Cla I
Sal I/Hinc II
Xho I
Apa I
Kpn I
hor:
e:
es:

Fig 33



Sac I, 6773
Hind III, 7
EcoR V, 219
Bgl II, 661
Sac II, 1213
Sac II, 1307
Apa I, 1364
Nde I, 1372
Cla I, 1552
Xho I, 1672
Hind III, 1692
Kpn I, 1872
Hinc II, 1897
Nde I, 2172
Sac I, 2272
Sac I, 2372
Bgl II, 2472
Nde I, 3382
maize 10 KD zein promoter
MSTS 1 TP
MSTS Ia
pESX 62
6773 bp
MCS
Pst I
Spe I
Xba I
Not I
EcoR I
Cla I
Sac II
Kpn I
Sac I
Sal I
Hinc II
Pst I
Amp
nos 3' terminator
MCS
Bgl II
Xba I
Hind III
Cla I
Sal I/Hinc II
Xho I
Apa I
Kpn I
Author:
Date:
Notes:

Fig 34

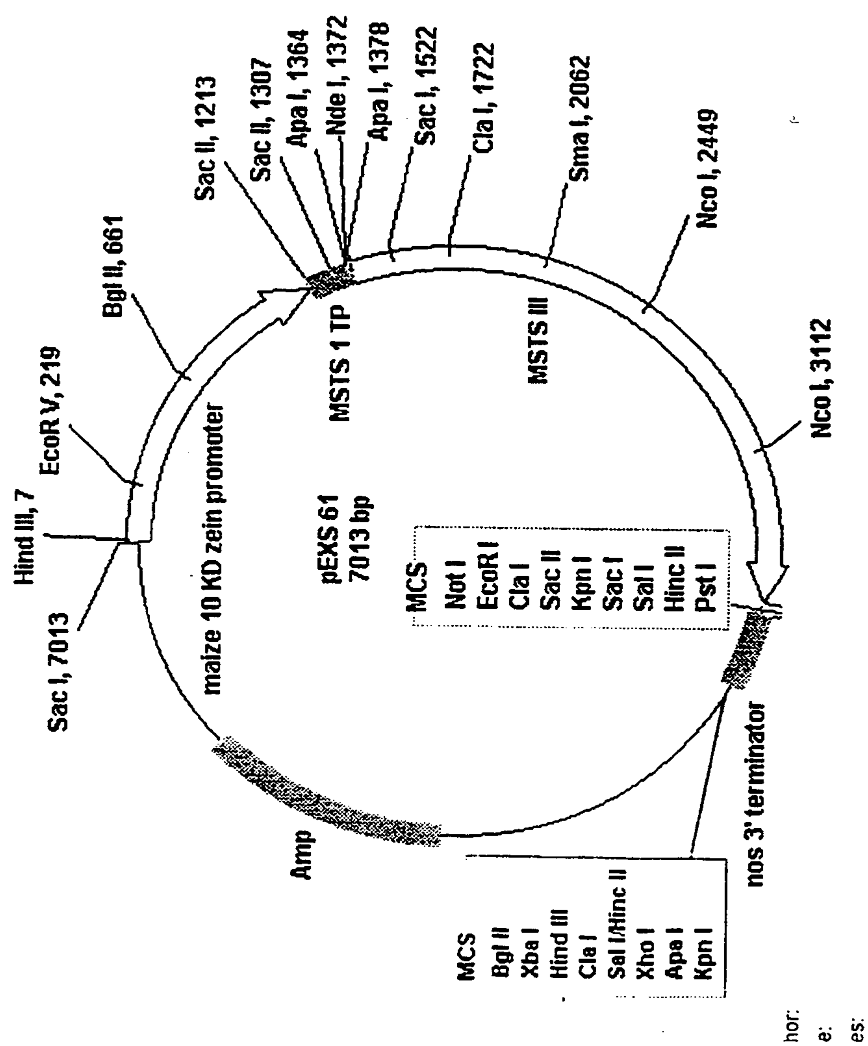
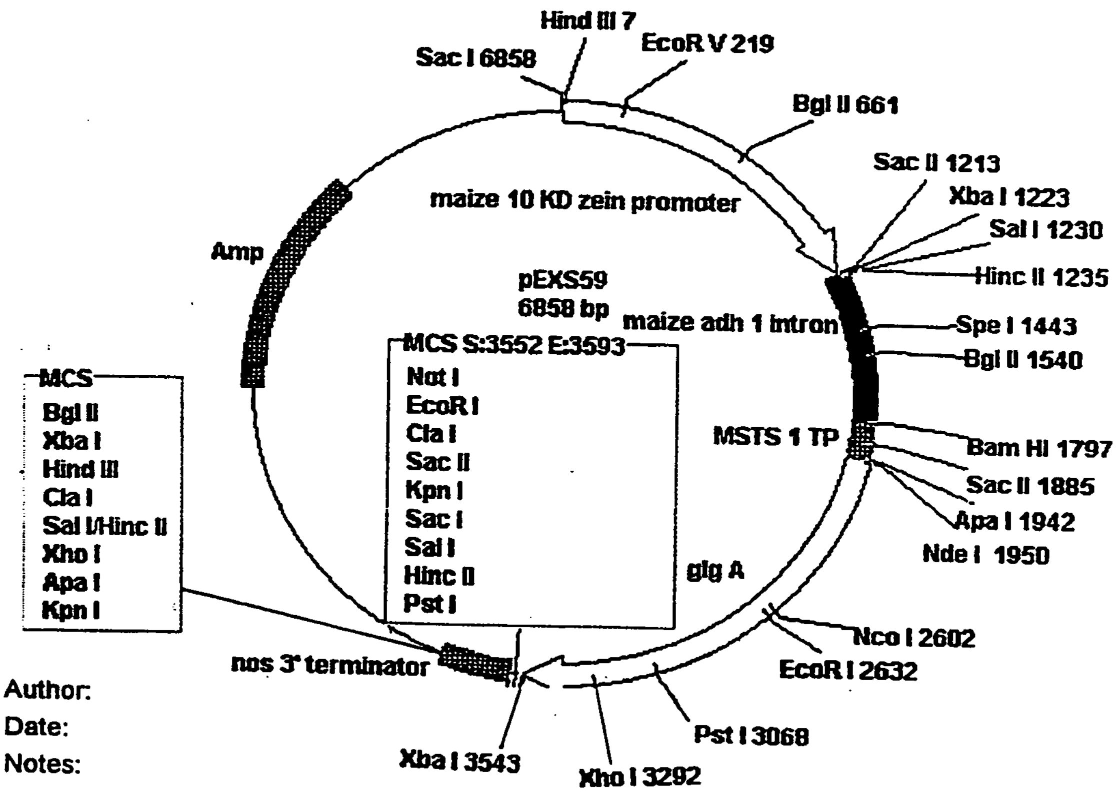
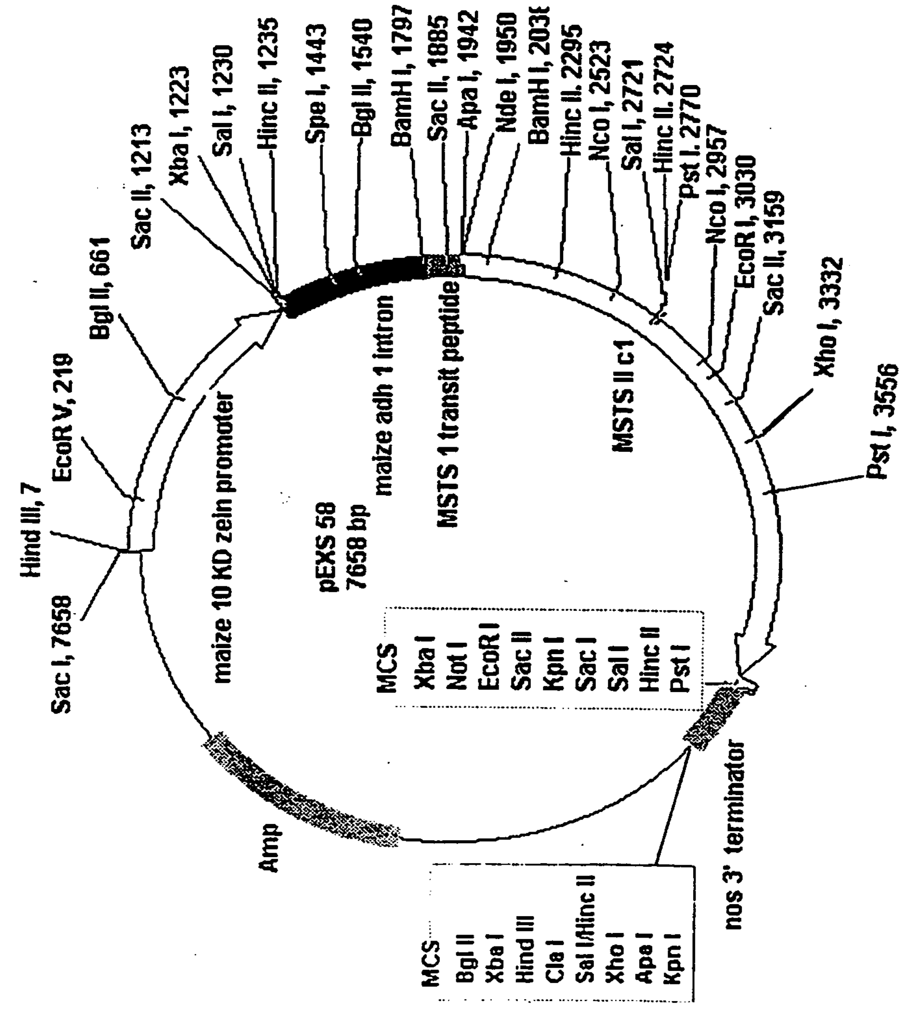

Sac II, 1213
Sac II, 1307
Apa I, 1364
Nde I, 1372
Apa I, 1378
Sac I, 1522
Cla I, 1722
Sma I, 2062
Nco I, 2449
Nco I, 3112
Bgl II, 661
EcoR V, 219
Hind III, 7
Sac I, 7013
MSTS 1 TP
MSTS III
maize 10 KD zein promoter
pEXS 61
7013 bp
MCS
Not I
EcoR I
Cla I
Sac II
Kpn I
Sac I
Sal I
Hinc II
Pst I
nos 3' terminator
Amp
MCS
Bgl II
Xba I
Hind III
Cla I
Sal I/Hinc II
Xho I
Apa I
Kpn I
hor:
e:
es:

Fig 35



Author:
Date:
Notes:

Fig. 36



thor:
te:
tes:

Fig 37

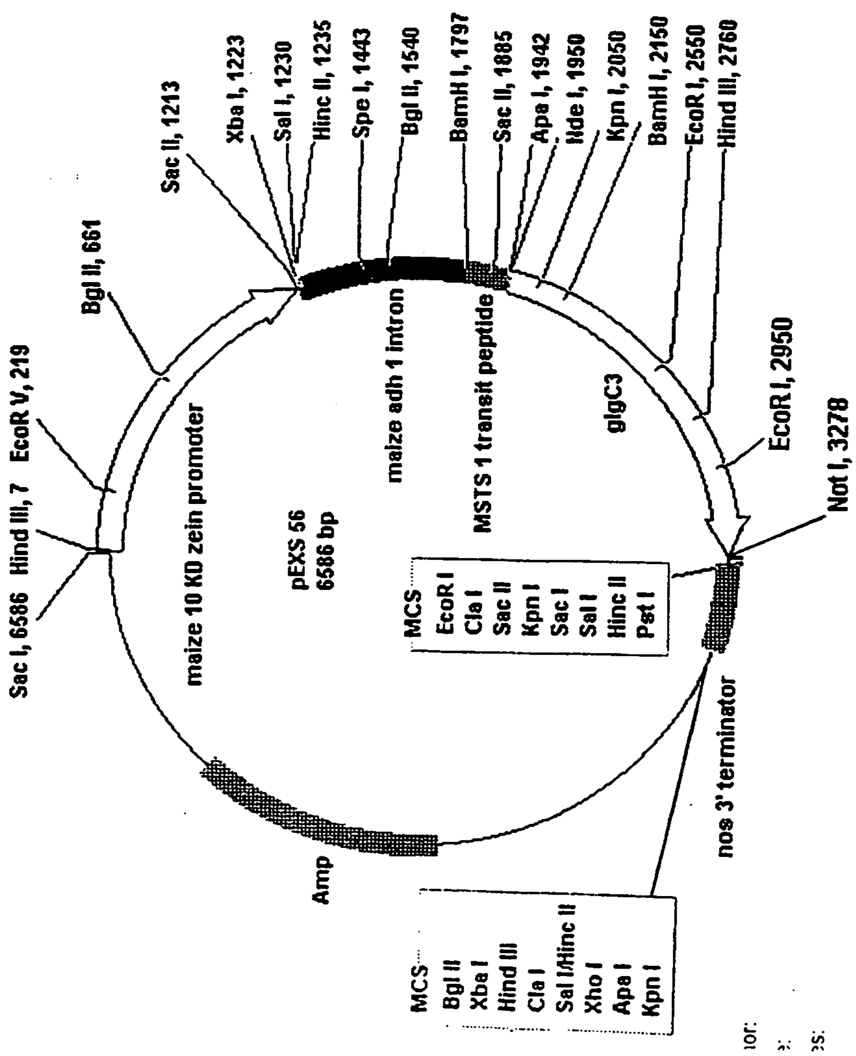
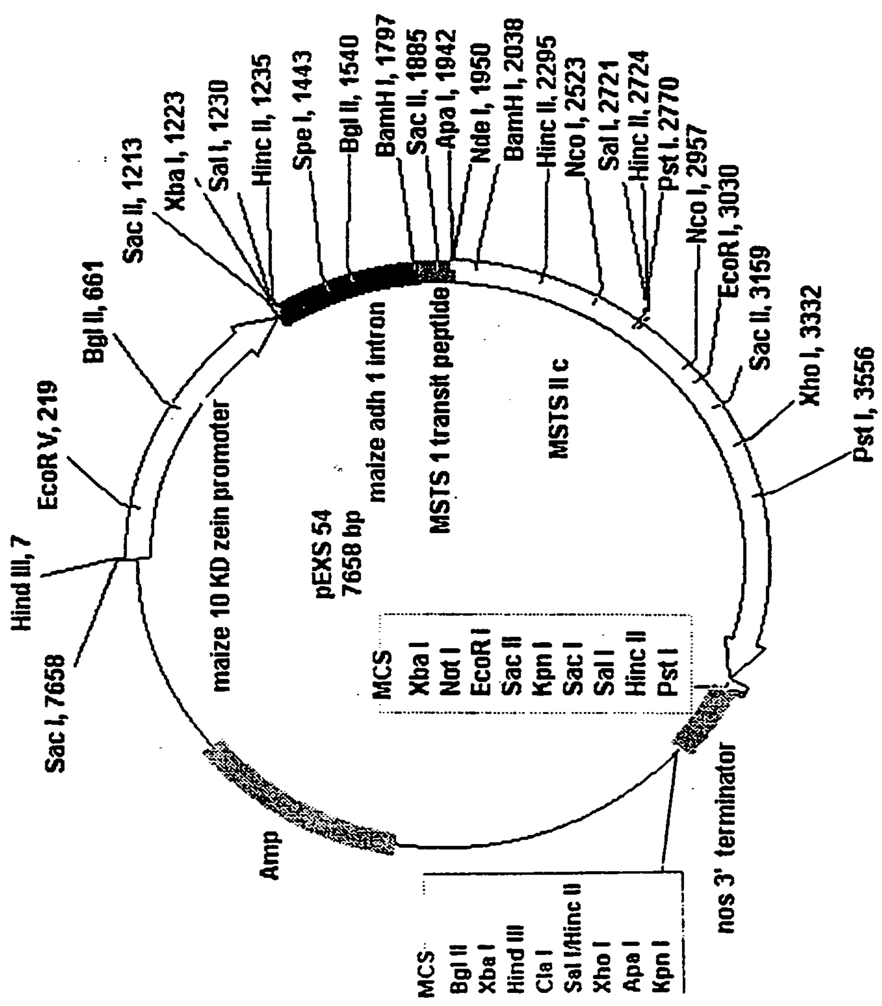

Sac I, 6586
Hind III, 7
EcoR V, 219
Bgl II, 661
Sac II, 1213
Xba I, 1223
Sal I, 1230
Hinc II, 1235
Spe I, 1443
Bgl II, 1540
BamH I, 1797
Sac II, 1885
Apa I, 1942
Nde I, 1950
Kpn I, 2050
BamH I, 2150
EcoR I, 2550
Hind III, 2760
EcoR I, 2950
Not I, 3278
maize 10 KD zein promoter
maize adh 1 intron
MSTS 1 transit peptide
glgC3
pEXS 56
6586 bp
MCS
EcoR I
Cla I
Sac II
Kpn I
Sac I
Sal I
Hinc II
Pst I
nos 3' terminator
Amp
MCS
Bgl II
Xba I
Hind III
Cla I
Sal I/Hinc II
Xho I
Apa I
Kpn I

Fig. 38


Hind III, 7
EcoR V, 219
Bgl II, 661
Sac II, 1213
Xba I, 1223
Sal I, 1230
Hinc II, 1235
Spe I, 1443
Bgl II, 1540
BamH I, 1797
Sac II, 1885
Apa I, 1942
Nde I, 1950
BamH I, 2038
Hinc II, 2295
Nco I, 2523
Sal I, 2721
Hinc II, 2724
Pst I, 2770
Nco I, 2957
EcoR I, 3030
Sac II, 3159
Xho I, 3332
Pst I, 3556
Sac I, 7658
maize 10 KD zein promoter
maize adh 1 intron
MSTS 1 transit peptide
MSTS II c
pEXS 54
7658 bp
MCS
Xba I
Not I
EcoR I
Sac II
Kpn I
Sac I
Sal I
Hinc II
Pst I
Amp
nos 3' terminator
MCS
Bgl II
Xba I
Hind III
Cla I
Sal I/Hinc II
Xho I
Apa I
Kpn I

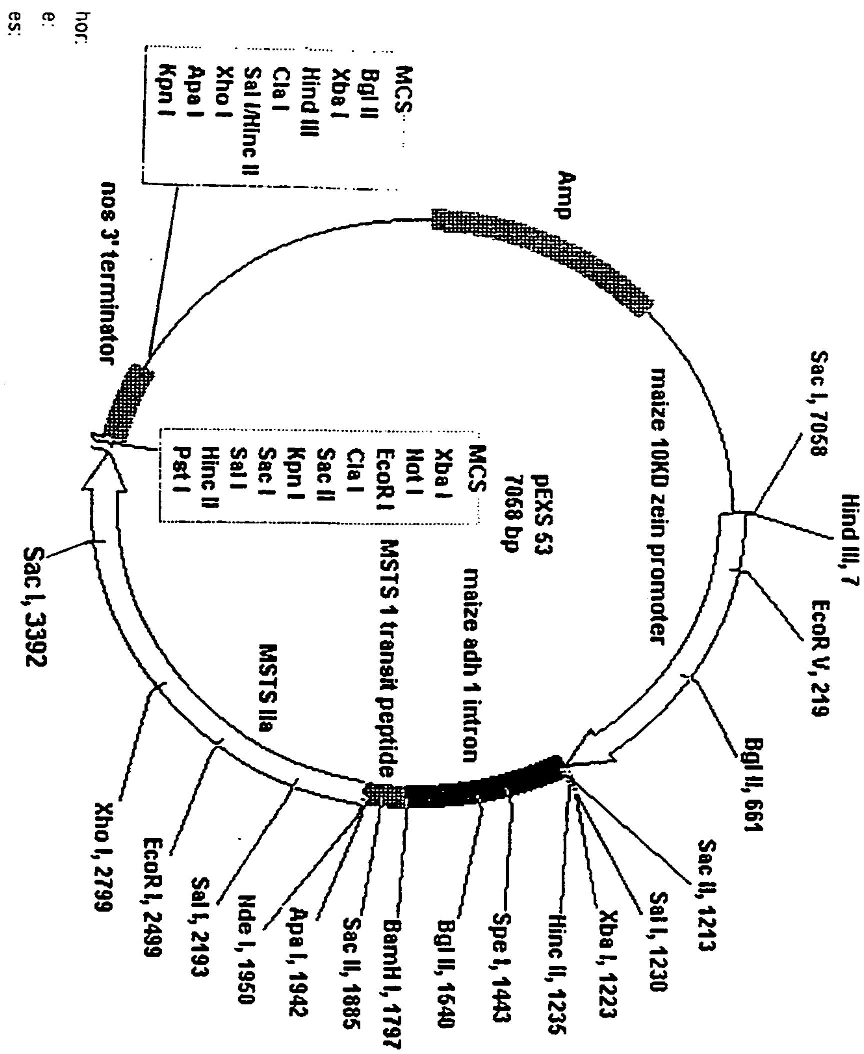

pEXS 53
7068 bp
Amp
maize 10KD zein promoter
maize adh 1 intron
MSTS 1 transit peptide
MSTS IIa
nos 3' terminator
MCS
Bgl II
Xba I
Hind III
Cla I
Sal I/Hinc II
Xho I
Apa I
Kpn I
MCS
Xba I
Not I
EcoR I
Cla I
Sac II
Kpn I
Sac I
Sal I
Hinc II
Pst I
Sac I, 7058
Hind III, 7
EcoR V, 219
Bgl II, 661
Sac II, 1213
Sal I, 1230
Xba I, 1223
Hinc II, 1235
Spe I, 1443
Bgl II, 1640
BamH I, 1797
Sac II, 1885
Apa I, 1942
Nde I, 1950
Sal I, 2193
EcoR I, 2499
Xho I, 2799
Sac I, 3392

Fig. 39

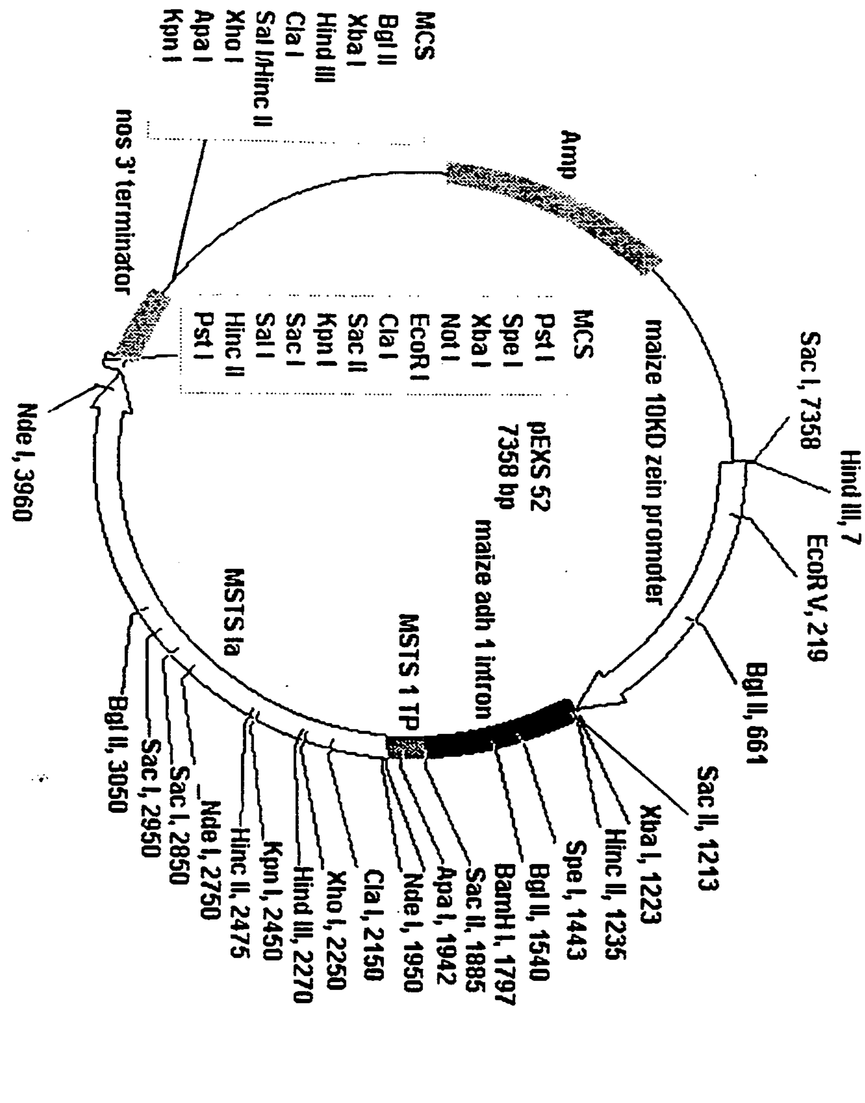

pEXS 52
7358 bp
Amp
maize 10KD zein promoter
maize adh 1 intron
MSTS 1 TP
MSTS Ia
nos 3' terminator
MCS
Pst I
Spe I
Xba I
Not I
EcoR I
Cla I
Sac II
Kpn I
Sac I
Sal I
Hinc II
Pst I
MCS
Bgl II
Xba I
Hind III
Cla I
Sal I/Hinc II
Xho I
Apa I
Kpn I
Sac I, 7358
Hind III, 7
EcoR V, 219
Bgl II, 661
Sac II, 1213
Xba I, 1223
Hinc II, 1235
Spe I, 1443
Bgl II, 1540
BamH I, 1797
Sac II, 1885
Apa I, 1942
Nde I, 1950
Cla I, 2150
Xho I, 2250
Hind III, 2270
Kpn I, 2450
Hinc II, 2475
Nde I, 2750
Sac I, 2850
Sac I, 2950
Bgl II, 3050
Nde I, 3960

Fig. 40

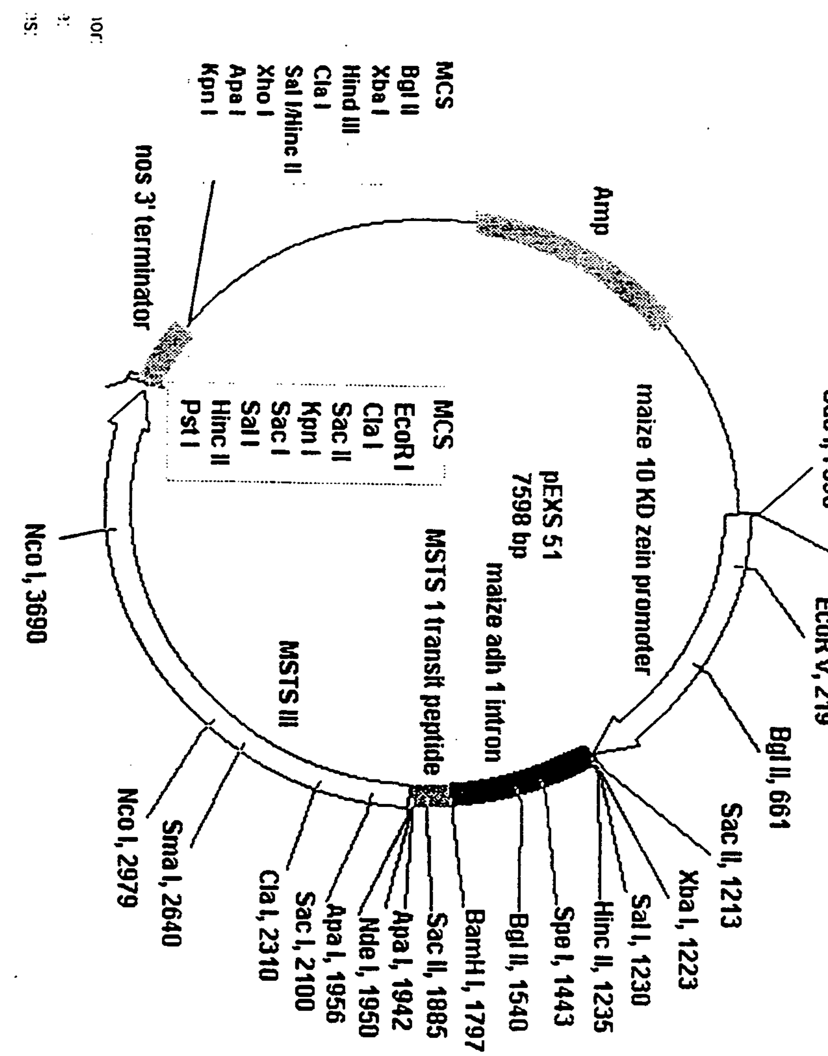

pEXS 51
7598 bp
Amp
maize 10 KD zein promoter
maize adh 1 intron
MSTS 1 transit peptide
MSTS III
nos 3' terminator
MCS
EcoR I
Cla I
Sac II
Kpn I
Sac I
Sal I
Hinc II
Pst I
MCS
Bgl II
Xba I
Hind III
Cla I
Sal I/Hinc II
Xho I
Apa I
Kpn I
Sac I, 7598
EcoR V, 219
Bgl II, 661
Sac II, 1213
Xba I, 1223
Sal I, 1230
Hinc II, 1235
Spe I, 1443
Bgl II, 1540
BamH I, 1797
Sac II, 1885
Apa I, 1942
Nde I, 1950
Apa I, 1956
Sac I, 2100
Cla I, 2310
Sma I, 2640
Nco I, 2979
Nco I, 3690

Fig 41

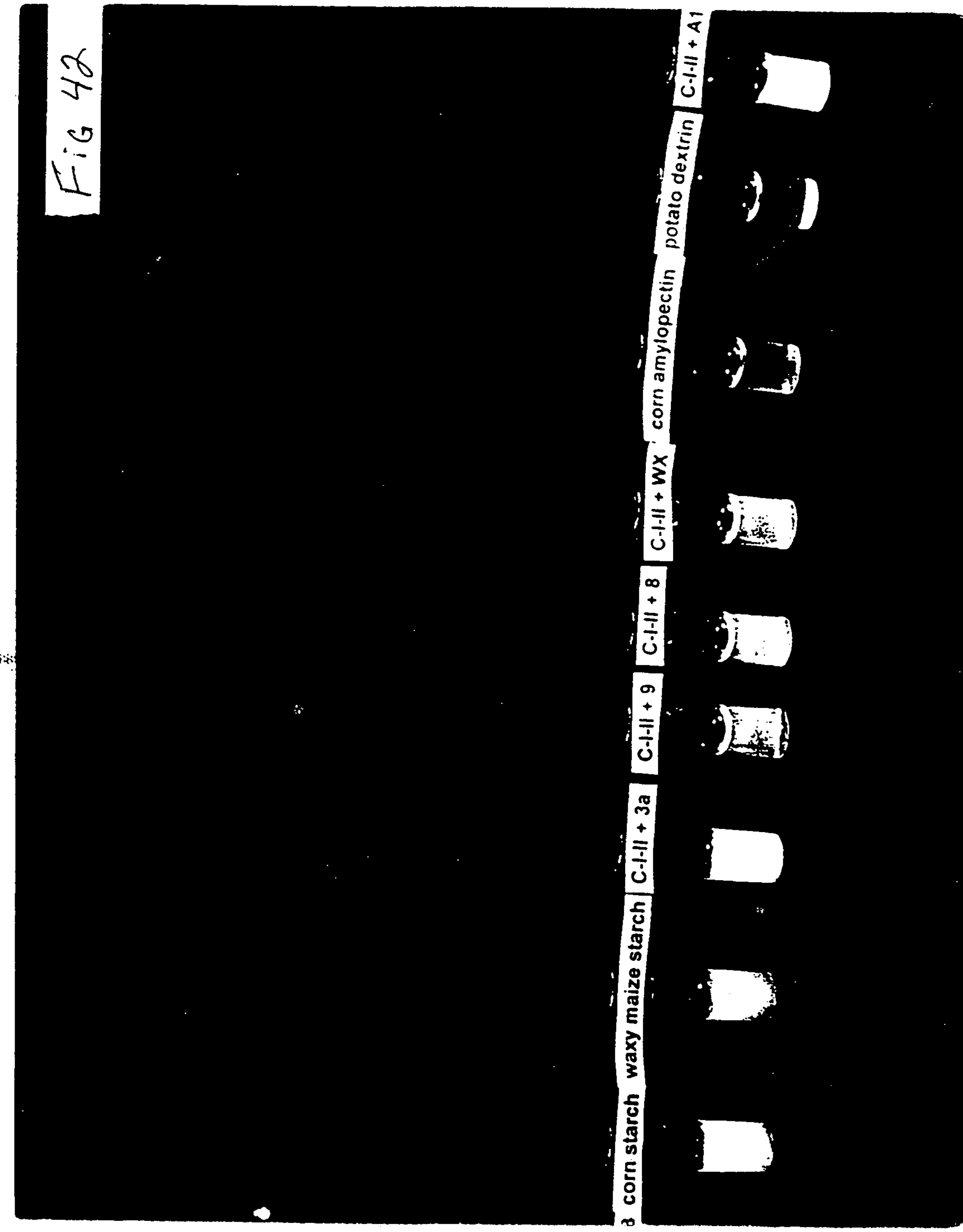

C-I-II + A1
potato dextrin
corn amylopectin
C-I-II + WX
C-I-II + 8
C-I-II + 9
C-I-II + 3a
waxy maize starch
corn starch

Fig 42

Fig 43

DNASIS ***** DNA TRANSLATION LIST ***** DATE 04-03-97

*** INPUT INFORMATION ***

FILE NAME : ECGLGA1.SEQ SEQUENCE : NORMAL 1488 BP

CODON TABLE : UNIV.TCN

SEQUENCE REGION : 1 - 1488

TRANSLATION REGION : 1 - 1488

*** DNA TRANSLATION ***

1	ATG	CAG	GTT	TTA	CAT	GTA	TGT	TCA	GAG	ATG	TTC	CCG	CTG	CTT	AAA	ACC	48
1	M	Q	V	L	H	V	C	S	E	M	F	P	L	L	K	T	16
49	GGC	GGT	CTG	GCT	GAT	GTT	ATT	GGG	GCA	TTA	CCC	GCA	GCA	CAA	ATC	GCA	96
17	G	G	L	A	D	V	I	G	A	L	P	A	A	Q	I	A	32
97	GAC	GGC	GTT	GAC	GCT	CGC	GTA	CTG	TTG	CCT	GCA	TTT	CCC	GAC	ATT	CGC	144
33	D	G	V	D	A	R	V	L	L	P	A	F	P	D	I	R	48
145	CGT	GGC	GTG	ACC	GAT	GCG	CAG	GTA	GTA	TCC	CGT	CGT	GAT	ACC	TCC	GCC	192
49	R	G	V	T	D	A	Q	V	V	S	R	R	D	T	S	A	64
193	GGA	CAT	ATC	ACG	CTG	TTG	TTC	GGT	CAT	TAC	AAC	GGG	GTT	GGC	ATT	TAC	240
65	G	H	I	T	L	L	F	G	H	Y	N	G	V	G	I	Y	80
241	CTG	ATT	GAC	GCG	CCG	CAT	CTC	TAT	GAT	CGT	CCG	GGA	AGT	CCG	TAT	CAC	288
81	L	I	D	A	P	H	L	Y	D	R	P	G	S	P	Y	H	96
289	GAT	ACC	AAC	TTA	TTT	GTC	CAT	ACC	GAC	AAC	GTA	TTG	CGT	TTT	GCG	CTG	336
97	D	T	N	L	F	V	H	T	D	N	V	L	R	F	A	L	112
337	CTG	GGG	TGG	GTT	GGG	GCA	GAA	ATG	GCC	AGC	GGG	CTT	GAC	CCA	TTC	TGG	384
113	L	G	W	V	G	A	E	M	A	S	G	L	D	P	F	W	128
385	CGT	CCT	GAT	GTG	GTG	CAT	GCG	CAC	GAC	TGG	CAT	GCA	GGC	CTT	GCG	CCT	432
129	R	P	D	V	V	H	A	H	D	W	H	A	G	L	A	P	144
433	GCG	TAT	CTG	GCG	GCG	CGC	GGG	CGT	CCG	GCG	AAG	TCG	GTG	TTT	ACT	GTG	480
145	A	Y	L	A	A	R	G	R	P	A	K	S	V	F	T	V	160
481	CAC	AAC	CTA	GCC	TAT	CAA	GGC	ATG	TTT	TAT	GCA	CAT	CAC	ATG	AAT	GAC	528
161	H	N	L	A	Y	Q	G	M	F	Y	A	H	H	M	N	D	176
529	ATC	CAA	TTG	CCA	TGG	TCA	TTC	TTT	AAT	ATT	CAT	GGG	CTG	GAA	TTC	AAC	576
177	I	Q	L	P	W	S	F	F	N	I	H	G	L	E	F	N	192
577	GGA	CAA	ATC	TCT	TTC	CTG	AAG	GCC	GGT	CTG	TAC	TAT	GCC	GAT	CAC	ATT	624
193	G	Q	I	S	F	L	K	A	G	L	Y	Y	A	D	H	I	208
625	ACG	GCG	GTC	AGT	CCA	ACC	TAC	GCT	CGC	GAG	ATC	ACC	GAA	CCG	CAG	TTT	672
209	T	A	V	S	P	T	Y	A	R	E	I	T	E	P	Q	F	224

44/90 Fig 43 continued

673 GCC TAC GGT ATG GAA GGT CTG TTG CAA CAG CGT CAC CGC GAA GGG CGT 720
225 A Y G M E G L L Q Q R H R E G R 240

721 CTT TCC GGC GTA CCG AAC GGC GTG GAC GAG AAA ATC TGG AGT CCA GAG 768
241 L S G V P N G V D E K I W S P E 256

769 ACG GAC TTA CTG TTG GCC TCG CGT TAC ACC CGC GAT ACG TTG GAA GAT 816
257 T D L L L A S R Y T R D T L E D 272

817 AAA GCG GAA AAT AAG CGC CAG TCA CAA ATC GCA ATG GGA TCC AAG GTT 864
273 K A E N K R Q S Q I A M G S K V 288

865 GAC GAT AAA GTG CCG CTT TTT GCA GTG GTG AGC CGT CTG ACC AGC CAG 912
289 D D K V P L F A V V S R L T S Q 304

913 AAA GGT CTC GAT TCG GTG CTG GAA GCC TCA CCG GGT TCT TCG GAG CAG 960
305 K G L D S V L E A S P G S S E Q 320

961 GGC GGG CAG CTG GCG CTA CTC GGC GCG GGC GAT CCG GTG CTG CAG GAA 1008
321 G G Q L A L L G A G D P V L Q E 336

1009 GGT TTC CTT GCG GCG GCA GCG GAA TAC CCC GGT CAG GTG GGC GTT CAG 1056
337 G F L A A A A E Y P G Q V G V Q 352

1057 ATT GGC TAT CAC GAA GCA TTT TCG CAT CGC ATT ATG GGC GGC GCG GAC 1104
353 I G Y H E A F S H R I M G G A D 368

1105 GTC ATT CTG GTG CCC AGC CGT TTC GAA CCG TGC GGC TTA ACG CAA CTT 1152
369 V I L V P S R F E P C G L T Q L 384

1153 TAT GGA TCG AAG TAC GGT ACG CTG CCG TTA GTG CGA CGC ACC GGT GGG 1200
385 Y G S K Y G T L P L V R R T G G 400

1201 CTT GCT GAT ACG GTT TCT GAC TGT TCT CTC GAG AAC CTT GCA GAT GGC 1248
401 L A D T V S D C S L E N L A D G 416

1249 GTC GCC AAT GGG TTT ATC TTC GAA GAT AGT AAT GCC TGG TCG CTG TTA 1296
417 V A N G F I F E D S N A W S L L 432

1297 CGG ACT ATT CGA CGT GCT TTT GTA CTG TGG TCC TGT CCT CCA CTG TGG 1344
433 R T I R R A F V L W S C P P L W 448

1345 CGG TTT GTG CAA CGT CAG GCT ATG GCA ATG GAT TTT GGC TGG CAG GTC 1392
449 R F V Q R Q A M A M D F G W Q V 464

1393 GCG GCG AAG TCG TAC CGT GAG CTT TAC TAT CGC TCG AAA TAG TTT TCA 1440
465 A A K S Y R E L Y Y R S K * F S 480

1441 GGA AAC GCC TAC ATG AAT GCT CCG TTT ACA TAT TCA TCG CCC ACG CTT 1488
481 G N A Y M N A P F T Y S S P T L 496

DNASIS ***** DNA TRANSLATION LIST ***** DATE 04-03-97

Fig. 44

*** INPUT INFORMATION ***

FILE NAME : ECGLGB.SEQ SEQUENCE : NORMAL 2361 BP

CODON TABLE : UNIV.TCN

SEQUENCE REGION : 1 - 2361

TRANSLATION REGION : 1 - 2361

*** DNA TRANSLATION ***

```
  1  ATG TCC GAT CGT ATC GAT AGA GAC GTG ATT AAC GCG CTA ATT GCA GGC   48
  1   M   S   D   R   I   D   R   D   V   I   N   A   L   I   A   G   16

 49  CAT TTT GCG GAT CCT TTT TCC GTA CTG GGA ATG CAT AAA ACC ACC GCG   96
 17   H   F   A   D   P   F   S   V   L   G   M   H   K   T   T   A   32

 97  GGA CTG GAA GTC CGT GCC CTT TTA CCC GAC GCT ACC GAT GTG TGG GTG  144
 33   G   L   E   V   R   A   L   L   P   D   A   T   D   V   W   V   48

145  ATT GAA CCG AAA ACC GGG CGC AAA CTC GCA AAA CTG GAG TGT CTC GAC  192
 49   I   E   P   K   T   G   R   K   L   A   K   L   E   C   L   D   64

193  TCA CGG GGA TTC TTT AGC GGC GTC ATT CCG CGA CGT AAG AAT TTT TTC  240
 65   S   R   G   F   F   S   G   V   I   P   R   R   K   N   F   F   80

241  CGC TAT CAG TTG GCT GTT GTC TGG CAT GGT CAG CAA AAC CTG ATT GAT  288
 81   R   Y   Q   L   A   V   V   W   H   G   Q   Q   N   L   I   D   96

289  GAT CCT TAC CGT TTT GGT CCG CTA ATC CAG GAA ATG GAT GCC TGG CTA  336
 97   D   P   Y   R   F   G   P   L   I   Q   E   M   D   A   W   L  112

337  TTA TCT GAA GGT ACT CAC CTG CGC CCG TAT GAA ACC TTA GGC GCG CAT  384
113   L   S   E   G   T   H   L   R   P   Y   E   T   L   G   A   H  128

385  GCA GAT ACT ATG GAT GGC GTC ACA GGT ACG CGT TTC TCT GTC TGG GCT  432
129   A   D   T   M   D   G   V   T   G   T   R   F   S   V   W   A  144

433  CCA AAC GCC CGT CGG GTC TCG GTG GTT GGG CAA TTC AAC TAC TGG GAC  480
145   P   N   A   R   R   V   S   V   V   G   Q   F   N   Y   W   D  160

481  GGT CGC CGT CAC CCG ATG CGC CTG CGT AAA GAG AGC GGC ATC TGG GAA  528
161   G   R   R   H   P   M   R   L   R   K   E   S   G   I   W   E  176

529  CTG TTT ATC CCT GGG GCG CAT AAC GGT CAG CTC TAT AAA TAC GAG ATG  576
177   L   F   I   P   G   A   H   N   G   Q   L   Y   K   Y   E   M  192

577  ATT GAT GCC AAT GGC AAC TTG CGT CTG AAG TCC GAC CCT TAT GCC TTT  624
193   I   D   A   N   G   N   L   R   L   K   S   D   P   Y   A   F  208

625  GAA GCG CAA ATG CGC CCG GAA ACC GCG TCT CTT ATT TGC GGG CTG CCG  672
209   E   A   Q   M   R   P   E   T   A   S   L   I   C   G   L   P  224
```

46/90

Fig 44 continued

673	GAA	AAG	GTT	GTA	CAG	ACT	GAA	GAG	CGC	AAA	AAA	GCG	AAT	CAG	TTT	GAT	720
225	E	K	V	V	Q	T	E	E	R	K	K	A	N	Q	F	D	240
721	GCG	CCA	ATC	TCT	ATT	TAT	GAA	GTT	CAC	CTG	GGT	TCC	TGG	CGT	CGC	CAC	768
241	A	P	I	S	I	Y	E	V	H	L	G	S	W	R	R	H	256
769	ACC	GAC	AAC	AAT	TTC	TGG	TTG	AGC	TAC	CGC	GAG	CTG	GCC	GAT	CAA	CTG	816
257	T	D	N	N	F	W	L	S	Y	R	E	L	A	D	Q	L	272
817	GTG	CCT	TAT	GCT	AAA	TGG	ATG	GGC	TTT	ACC	CAC	CTC	GAA	CTA	CTG	CCC	864
273	V	P	Y	A	K	W	M	G	F	T	H	L	E	L	L	P	288
865	ATT	AAC	GAG	CAT	CCC	TTC	GAT	GGC	AGT	TGG	GGT	TAT	CAG	CCA	ACC	GGC	912
289	I	N	E	H	P	F	D	G	S	W	G	Y	Q	P	T	G	304
913	CTG	TAT	GCG	CCA	ACC	CGC	CGT	TTT	GGT	ACT	CGC	GAC	GAC	TTC	CGT	TAT	960
305	L	Y	A	P	T	R	R	F	G	T	R	D	D	F	R	Y	320
961	TTC	ATT	GAT	GCC	GCA	CAC	GCA	GCT	GGT	CTG	AAC	GTG	ATT	CTC	GAC	TGG	1008
321	F	I	D	A	A	H	A	A	G	L	N	V	I	L	D	W	336
1009	GTG	CCA	GGC	CAC	TTC	CCG	ACT	GAT	GAC	TTT	GCG	CTT	GCC	GAA	TTT	GAT	1056
337	V	P	G	H	F	P	T	D	D	F	A	L	A	E	F	D	352
1057	GGC	ACG	AAC	TTG	TAT	GAA	CAC	AGC	GAT	CCG	CGT	GAA	GGC	TAT	CAT	CAG	1104
353	G	T	N	L	Y	E	H	S	D	P	R	E	G	Y	H	Q	368
1105	GAC	TGG	AAC	ACG	CTG	ATC	TAC	AAC	TAT	GGT	CGC	CGT	GAA	GTC	AGT	AAC	1152
369	D	W	N	T	L	I	Y	N	Y	G	R	R	E	V	S	N	384
1153	TTC	CTC	GTC	GGT	AAC	GCG	CTT	TAC	TGG	ATT	GAA	CGT	TTT	GGT	ATT	GAT	1200
385	F	L	V	G	N	A	L	Y	W	I	E	R	F	G	I	D	400
1201	GCG	CTG	CGC	GTC	GAT	GCG	GTG	GCG	TCA	ATG	ATT	TAT	CGC	GAC	TAC	AGC	1248
401	A	L	R	V	D	A	V	A	S	M	I	Y	R	D	Y	S	416
1249	CGT	AAA	GAG	GGG	GAG	TGG	ATC	CCG	AAC	GAA	TTT	GGC	GGG	CGC	GAG	AAT	1296
417	R	K	E	G	E	W	I	P	N	E	F	G	G	R	E	N	432
1297	CTT	GAA	GCG	ATT	GAA	TTC	TTG	CGT	AAT	ACC	AAC	CGT	ATT	CTT	GGT	GAG	1344
433	L	E	A	I	E	F	L	R	N	T	N	R	I	L	G	E	448
1345	CAG	GTT	TCC	GGT	GCG	GTG	ACA	ATG	GCT	GAG	GAG	TCT	ACC	GAT	TTC	CCT	1392
449	Q	V	S	G	A	V	T	M	A	E	E	S	T	D	F	P	464
1393	GGC	GTT	TCT	CGT	CCG	CAG	GAT	ATG	GGC	GGT	CTG	GGC	TTC	TGG	TAC	AAG	1440
465	G	V	S	R	P	Q	D	M	G	G	L	G	F	W	Y	K	480
1441	TGG	AAC	CTC	GGC	TGG	ATG	CAT	GAC	ACC	TTG	GAC	TAC	ATG	AAG	CTC	GAC	1488
481	W	N	L	G	W	M	H	D	T	L	D	Y	M	K	L	D	496
1489	CCG	GTT	TAT	CGT	CAG	TAT	CAT	CAC	GAT	AAA	CTG	ACC	TTC	GGG	ATT	CTC	1536
497	P	V	Y	R	Q	Y	H	H	D	K	L	T	F	G	I	L	512
1537	TAC	AAC	TAC	ACT	GAA	AAC	TTC	GTC	CTG	CCG	TTG	TCG	CAT	GAT	GAA	GTG	1584
513	Y	N	Y	T	E	N	F	V	L	P	L	S	H	D	E	V	528
1585	GTC	CAC	GGT	AAA	AAA	TCG	ATT	CTC	GAC	CGC	ATG	CCG	GGC	GAC	GCA	TGG	1632
529	V	H	G	K	K	S	I	L	D	R	M	P	G	D	A	W	544

Fig 44 continued

1633	CAG	AAA	TTC	GCG	AAC	CTG	CGC	GCC	TAC	TAT	GGC	TGG	ATG	TGG	GCA	TTC	1680
545	Q	K	F	A	N	L	R	A	Y	Y	G	W	M	W	A	F	560
1681	CCG	GGC	AAG	AAA	CTA	CTG	TTC	ATG	GGT	AAC	GAA	TTT	GCC	CAG	GGC	CGC	1728
561	P	G	K	K	L	L	F	M	G	N	E	F	A	Q	G	R	576
1729	GAG	TGG	AAC	CAT	GAC	GCC	AGC	CTC	GAC	TGG	CAT	CTG	TTG	GAA	GGC	GGC	1776
577	E	W	N	H	D	A	S	L	D	W	H	L	L	E	G	G	592
1777	GAT	AAC	TGG	CAC	CAC	GGT	GTC	CAG	CGT	CTG	GTG	CGC	GAT	CTG	AAC	CTC	1824
593	D	N	W	H	H	G	V	Q	R	L	V	R	D	L	N	L	608
1825	ACC	TAC	CGC	CAC	CAT	AAA	GCA	ATG	CAT	GAA	CTG	GAT	TTT	GAC	CCG	TAC	1872
609	T	Y	R	H	H	K	A	M	H	E	L	D	F	D	P	Y	624
1873	GGC	TTT	GAA	TGG	CTG	GTG	GTG	GAT	GAC	AAA	GAA	CGC	TCG	GTG	CTG	ATC	1920
625	G	F	E	W	L	V	V	D	D	K	E	R	S	V	L	I	640
1921	TTT	GTG	CGT	CGC	GAT	AAA	GAG	GGT	AAC	GAA	ATC	ATC	GTT	GCC	AGT	AAC	1968
641	F	V	R	R	D	K	E	G	N	E	I	I	V	A	S	N	656
1969	TTT	ACG	CCG	GTA	CCG	CGT	CAT	GAT	TAT	CGC	TTC	GGC	ATA	AAC	CAG	CCG	2016
657	F	T	P	V	P	R	H	D	Y	R	F	G	I	N	Q	P	672
2017	GGC	AAA	TGG	CGT	GAA	ATC	CTC	AAT	ACC	GAT	TCC	ATG	CAC	TAT	CAC	GGC	2064
673	G	K	W	R	E	I	L	N	T	D	S	M	H	Y	H	G	688
2065	AGT	AAT	GCA	GGC	AAT	GGC	GGC	ACG	GTA	CAC	AGC	GAT	GAG	ATT	GCC	AGC	2112
689	S	N	A	G	N	G	G	T	V	H	S	D	E	I	A	S	704
2113	CAC	GGT	CGT	CAG	CAT	TCA	CTA	AGC	CTG	ACG	CTA	CCA	CCG	CTG	GCC	ACT	2160
705	H	G	R	Q	H	S	L	S	L	T	L	P	P	L	A	T	720
2161	ATC	TGG	CTG	GTT	CGG	GAG	GCA	GAA	TGA	CAC	AAC	TCG	CCA	TTG	GCA	AAC	2208
721	I	W	L	V	R	E	A	E	*	H	N	S	P	L	A	N	736
2209	CCG	CTC	CCC	TCG	GCG	CGC	ATT	ACG	ACG	GTC	AGG	GCG	TCA	ACT	TCA	CAC	2256
737	P	L	P	S	A	R	I	T	T	V	R	A	S	T	S	H	752
2257	TTT	TCT	CCG	CTC	ATG	CCG	AGC	GGG	TAG	AAC	TGT	GTG	TCT	TTG	ACG	CCA	2304
753	F	S	P	L	M	P	S	G	*	N	C	V	S	L	T	P	768
2305	ATG	GCC	AGG	AAC	ATC	GCT	ATG	ACT	TGC	CAG	GGC	ACA	GTG	GCG	ACA	TTT	2352
769	M	A	R	N	I	A	M	T	C	Q	G	T	V	A	T	F	784
2353	GGC	ACG	GTT														2361
785	G	T	V														787

Fig 45a

```
LOCUS       MZEZEIN10K   2562 bp    DNA             PLN       19-J
UL-1994
DEFINITION  Zea mays 10-kDa zein gene, complete cds.
ACCESSION   M23537
NID         g340933
KEYWORDS    methionine-rich protein; seed storage protein; zein pr
otein.
SOURCE      Zea mays 3-week old seedling leaf DNA.
  ORGANISM  Zea mays
            Eukaryota; Plantae; Embryobionta; Magnoliophyta; Lilio
psida;
            Commelinidae; Cyperales; Poaceae.
REFERENCE   1  (bases 1 to 2562)
  AUTHORS   Kirihara,J.A., Petri,J.B. and Messing,J.
  TITLE     Isolation and sequence of a gene encoding a methionine
-rich 10-kDa
            zein protein from maize
  JOURNAL   Gene 71, 359-370 (1988)
  MEDLINE   89138012
COMMENT     NCBI gi: 340933
FEATURES             Location/Qualifiers
     source          1. .2562
                     /organism="Zea mays"
                     /cell_line="inbred BSSS-53"
                     /dev_stage="3-week old seedling"
                     /sequenced_mol="DNA"
                     /tissue_type="leaf"
     TATA_signal     1044. .1050
     mRNA            1082. .1685
     CDS             1137. .1589
                     /note="10 kDa;  NCBI gi: 511870"
                     /codon_start=1
                     /product="zein protein"
                     /db_xref="PID:g511870"
                     /translation="MAAKMLALFALLALCASATSATHIPGHLPPV
MPLGTMNPCMQYC
                     MMQQGLASLMACPSLMLQQLLALPLQTMPVMMPQMMTPNMMSPLM
MPSMMSPMVLPSM
                     MSQIMMPQCHCDAVSQIMLQQQLPFMFNPMAMTIPPMFLQQPFVG
AAF"
     sig_peptide     1137. .1199
                     /codon_start=1
     mat_peptide     1200. .1586
                     /note="10 kDa"
                     /codon_start=1
                     /product="zein protein"
     polyA_signal    1655. .1660
BASE COUNT      782 a    506 c    471 g    803 t
ORIGIN

 MZEZEIN10K  Length: 2562  May 29, 1996 15:41  Type: N  Check: 840
```

~DSP0131.TMP

Fig 45a continued

8 ..

```
   1 AAGCTTGCTA CTTTCTTTCC TTAATGTTGA TTTCCCCTTT GTTAGATGTT
  51 CTTTGTGTTA TATACACTCT GTATACAAGG ATGCGATACA CACATCAGCT
 101 AGTCCTAATG ATGCCACCGA CTTTACTTGA GGAAAAGGAA ACAAATATGA
 151 TGTGGCCATC ACATTCTCAA TAACAATGAC CATGTGCGCA ATGACATACC
 201 ATCATATTTG ATATCATAAA AATAAATTTA TTATCAAAGT AAACATATAG
 251 TTCATATATC AGATATTAAA GTGATAAGAA CAAATATTAC ATTTTATCTT
 301 ATATAAAATG ACGAAAAAGG TACGAGTTGA AAAGGAGTCC AACCCCTTTT
 351 TTATAGCTTG TTCGGTTGCT TGTTCTCTTC GGCTAGCGAG GTGGTAGAAT
 401 GTGAGAGTGT TGCGCGTGGA TTCCCGTCGT AGTGTTCTTA GGTGATTTCT
 451 CACGGCCCAT CTGTGATATA GCGACTCATA TGTGGTGTAA TAGCCCATTG
 501 GGAGAAGGGG AGAGATATAG ATCTACGTGA TTTGCACGTG ATGCACGACG
 551 AACGAAACTG GTGGTTTAAA GTAGTAGAGG TTTGTCATTA GAGGTGTAAA
 601 TGGTACATAT ATTATCCGTT CATATTCGAA TTTGATCCGT ATAAGAGGGC
 651 TAAGATCTAA TCCGTATACA AGTCCAAGTA TTAAGTATCC GATCCATATC
 701 GGATCTTTAT CCGTATCCGT ATACTCAAAA TTTGATGTTT AAGATTTTAA
 751 TATATATTTA AACTTTATAG GAACTCGATA ATATTTGTAT CTGATTTGAA
 801 TTATGAAAAC AAATATGGAA CGATTAATTT CAGTCTATAT CCGTTCCGAT
 851 ATTTGTCATG CTTTGCTAAA AATACCTTTA CAAGGCATCT TGTGCAGATT
 901 ATATATTAAT CTGAAATCAG TTAGAGAAGC CTACAAATTT GACCAAATGC
 951 CGAGTCATCC GGCTTATCCC CTTTCCAACT TTCAGTTCTG CAAGCGCCAG
1001 AAATCGTTTT TCATCTACAT TGTCTTTGTT GCCTGCATAC ATCTATAAAT
1051 AGGACCTGCT AGATCAATCG CAGTCCATCG GCCTCAGTCG CACATATCTA
1101 CTATACTATA CTCTAGGAAG CAAGGACACC ACCGCCATGG CAGCCAAGAT
1151 GCTTGCATTG TTCGCTCTCC TAGCTCTTTG TGCAAGCGCC ACTAGTGCGA
1201 CCCATATTCC AGGGCACTTG CCACCAGTCA TGCCATTGGG TACCATGAAC
```

~DSP0131.TMP

Fig 45a continued

1251 CCATGCATGC AGTACTGCAT GATGCAACAG GGGCTTGCCA GCTTGATGGC
1301 GTGTCCGTCC CTGATGCTGC AGCAACTGTT GGCCTTACCG CTTCAGACGA
1351 TGCCAGTGAT GATGCCACAG ATGATGACGC CTAACATGAT GTCACCATTG
1401 ATGATGCCGA GCATGATGTC ACCAATGGTC TTGCCGAGCA TGATGTCGCA
1451 AATAATGATG CCACAATGTC ACTGCGACGC CGTCTCGCAG ATTATGCTGC
1501 AACAGCAGTT ACCATTCATG TTCAACCCAA TGGCCATGAC GATTCCACCC
1551 ATGTTCTTAC AGCAACCCTT TGTTGGTGCT GCATTCTAGA TAGAAATATT
1601 TGTGTTGTAT CGAATAATGA GTTGACATGC CATCGCGTGT GACTCATTAT
1651 TAACAATAAA ACAAGTTTCC TCTTATTATC TTTTTATATC TCTCCCTATC
1701 CATTTTTGCA AAGCCCATTA TCCTTTACTC CCTAAGTCCC AATATATTTT
1751 AGACCTTAAA TTGTATGTCT ATATTCAAAA GAATGACAAT AAATCTAGAC
1801 ATATATATAA AACACATACA TTAAGTATTG TATGAATCTA TTAAAATGCT
1851 AAAACGACTA ATATTATGGG ACGGAGGGAG TACTTTATTA GTAGATTACA
1901 TTGTTATTTT CTCTATTCCA AATATAAGTC TGGTTTTTCA ATCAATCAAT
1951 ATATATTACC ATGTCCAAAC ATTTTGAATT ATATATCTAG GTGCAGCATC
2001 CGTGCACGAT CGTAAAAGAA GCAGTCACGG TGTTGGTCCC AAAAACTAAT
2051 CGTCCGTTGT CGGTCACCTA TAAAGATTCA TGAAGAGAAC CAAAATAAGG
2101 CAATATAATT AATGTAATAT GACTCCTCCT TTTGAATTAC TTAGGAATAA
2151 CATAAGCAAA CAAAAAAAGG AGAAGATCAA GGTAAATAAA GGCATTTTGT
2201 GAGAAAACAT GGAAGCATAA GAATGCATAA GTAATGATTT GTGTCTCTTT
2251 ATATTTTTTT TATTCACGTG AATTTACATA GATACCATCG GATGTTCGAT
2301 GGTAATACAA TGATGCCTTA GCTCCGAGAG CTTCGAATGA TGAGCGATTT
2351 AAAAATACTC CTATCAATTG TTCGAAAGTT CTTTGTCTCA TGCATGGGCA
2401 ATGTACCTCT ATTTATAGGG ACGGTGCGAC GTACAAATTT GTATAAAATT
2451 ATATTTTTAT TCCCAAATCC TATGCATATG TGTCGGGGAC CATAATTAGG

~DSP0131.TMP

Fig 45a continued

2501 GGTACCCTCA AGGCTCCTAA TTCTCAGCTG GTAACCCCAT CAGCATAAAG

2551 CTGCAAAGGC CT

Fig. 45b

```
LOCUS       MZEZEIN10K  2562 bp   DNA          PLN       19-JUL-1994
DEFINITION  Zea mays 10-kDa zein gene, complete cds.
ACCESSION   M23537
NID         g340933
KEYWORDS    methionine-rich protein; seed storage protein; zein protein.
SOURCE      Zea mays 3-week old seedling leaf DNA.
  ORGANISM  Zea mays
            Eukaryota; Plantae; Embryobionta; Magnoliophyta; Liliopsida;
            Commelinidae; Cyperales; Poaceae.
REFERENCE   1  (bases 1 to 2562)
  AUTHORS   Kirihara,J.A., Petri,J.B. and Messing,J.
  TITLE     Isolation and sequence of a gene encoding a methionine-rich 10-kDa
            zein protein from maize
  JOURNAL   Gene 71, 359-370 (1988)
  MEDLINE   89138012
COMMENT     NCBI gi: 340933
FEATURES             Location/Qualifiers
     source          1..2562
                     /organism="Zea mays"
                     /cell_line="inbred BSSS-53"
                     /dev_stage="3-week old seedling"
                     /sequenced_mol="DNA"
                     /tissue_type="leaf"
     TATA_signal     1044..1050
     mRNA            1082..1685
     CDS             1137..1589
                     /note="10 kDa; NCBI gi: 511870"
                     /codon_start=1
                     /product="zein protein"
                     /db_xref="PID:g511870"
/translation="MAAKMLALFALLALCASATSATHIPGHLPPVMPLGTMNPCMQYC
MMQQGLASLMACPSLMLQQLLALPLQTMPVMMPQMMTPNMMSPLMMPSMMSPMVL
PSM
                     MSQIMMPQCHCDAVSQIMLQQQLPFMFNPMAMTIPPMFLQQPFVGAAF"
     sig_peptide     1137..1199
                     /codon_start=1
     mat_peptide     1200..1586
                     /note="10 kDa"
                     /codon_start=1
                     /product="zein protein"
     polyA_signal    1655..1660
BASE COUNT      782 a    506 c    471 g    803 t
ORIGIN
```

Fig 45b continued

MZEZEIN10K Length: 2562 May 29, 1996 15:41 Type: N Check: 8408 ..

```
   1 AAGCTTGCTA CTTTCTTTCC TTAATGTTGA TTTCCCCTTT GTTAGATGTT
  51 CTTTGTGTTA TATACACTCT GTATACAAGG ATGCGATACA CACATCAGCT
 101 AGTCCTAATG ATGCCACCGA CTTTACTTGA GGAAAAGGAA ACAAATATGA
 151 TGTGGCCATC ACATTCTCAA TAACAATGAC CATGTGCGCA ATGACATACC
 201 ATCATATTTG ATATCATAAA AATAAATTTA TTATCAAAGT AAACATATAG
 251 TTCATATATC AGATATTAAA GTGATAAGAA CAAATATTAC ATTTTATCTT
 301 ATATAAAATG ACGAAAAAGG TACGAGTTGA AAAGGAGTCC AACCCCTTTT
 351 TTATAGCTTG TTCGGTTGCT TGTTCTCTTC GGCTAGCGAG GTGGTAGAAT
 401 GTGAGAGTGT TGCGCGTGGA TTCCCGTCGT AGTGTTCTTA GGTGATTTCT
 451 CACGGCCCATCTGTGATATAGCGACTCATATATGTGGTGTAATAGCCCATTG
 501 GGAGAAGGGG AGAGATATAG ATCTACGTGA TTTGCACGTG ATGCACGACG
 551 AACGAAACTG GTGGTTTAAA GTAGTAGAGG TTTGTCATTA GAGGTGTAAA
 601 TGGTACATAT ATTATCCGTT CATATTCGAA TTTGATCCGT ATAAGAGGGC
 651 TAAGATCTAA TCCGTATACA AGTCCAAGTA TTAAGTATCC GATCCATATC
 701 GGATCTTTAT CCGTATCCGT ATACTCAAAA TTTGATGTTT AAGATTTTAA
 751 TATATATTTA AACTTTATAG GAACTCGATA ATATTTGTAT CTGATTTGAA
 801 TTATGAAAAC AAATATGGAA CGATTAATTT CAGTCTATAT CCGTTCCGAT
 851 ATTTGTCATG CTTTGCTAAA AATACCTTTA CAAGGCATCT TGTGCAGATT
 901 ATATATTAAT CTGAAATCAG TTAGAGAAGC CTACAAATTT GACCAAATGC
 951 CGAGTCATCC GGCTTATCCC CTTTCCAACT TTCAGTTCTG CAAGCGCCAG
1001 AAATCGTTTT TCATCTACAT TGTCTTTGTT GCCTGCATAC ATCTATAAAT
1051 AGGACCTGCT AGATCAATCG CAGTCCATCG GCCTCAGTCG CACATATCTA
1101 CTATACTATA CTCTAGGAAG CAAGGACACC ACCGCCATG
```

Fig. 46

DNASIS ***** DNA TRANSLATION LIST ***** DATE 04-03-97

*** INPUT INFORMATION ***

FILE NAME : ECGLGC3.SEQ SEQUENCE : NORMAL 1328 BP

CODON TABLE : UNIV.TCN

SEQUENCE REGION : 1 - 1328

TRANSLATION REGION : 1 - 1326

*** DNA TRANSLATION ***

1	ATG	GTT	AGT	TTA	GAG	AAG	AAC	GAT	CAC	TTA	ATG	TTG	GCG	CGC	CAG	CTG	48
1	M	V	S	L	E	K	N	D	H	L	M	L	A	R	Q	L	16
49	CCA	TTG	AAA	TCT	GTT	GCC	CTG	ATA	CTG	GCG	GGA	GGA	CGT	GGT	ACC	CGC	96
17	P	L	K	S	V	A	L	I	L	A	G	G	R	G	T	R	32
97	CTG	AAG	GAT	TTA	ACC	AAT	AAG	CGA	GCA	AAA	CCG	GCC	GTA	CAC	TTC	GGC	144
33	L	K	D	L	T	N	K	R	A	K	P	A	V	H	F	G	48
145	GGT	AAG	TTC	CGC	ATT	ATC	GAC	TTT	GCG	CTG	TCT	AAC	TGC	ATC	AAC	TCC	192
49	G	K	F	R	I	I	D	F	A	L	S	N	C	I	N	S	64
193	GGG	ATC	CGT	CGT	ATG	GGC	GTG	ATC	ACC	CAG	TAC	CAG	TCC	CAC	ACT	CTG	240
65	G	I	R	R	M	G	V	I	T	Q	Y	Q	S	H	T	L	80
241	GTG	CAG	CAC	ATT	CAG	CGC	GGC	TGG	TCA	TTC	TTC	AAT	GAA	GAA	ATG	AAC	288
81	V	Q	H	I	Q	R	G	W	S	F	F	N	E	E	M	N	96
289	GAG	TTT	GTC	GAT	CTG	CTG	CCA	GCA	CAG	CAG	AGA	ATG	AAA	GGG	GAA	AAC	336
97	E	F	V	D	L	L	P	A	Q	Q	R	M	K	G	E	N	112
337	TGG	TAT	CGC	GGC	ACC	GCA	GAT	GCG	GTC	ACC	CAA	AAC	CTC	GAC	ATT	ATC	384
113	W	Y	R	G	T	A	D	A	V	T	Q	N	L	D	I	I	128
385	CGT	CGT	TAT	AAA	GCG	GAA	TAC	GTG	GTG	ATC	CTG	GCG	GGC	GAC	CAT	ATC	432
129	R	R	Y	K	A	E	Y	V	V	I	L	A	G	D	H	I	144
433	TAC	AAG	CAA	GAC	TAC	TCG	CGT	ATG	CTT	ATC	GAT	CAC	GTC	GAA	AAA	GGT	480
145	Y	K	Q	D	Y	S	R	M	L	I	D	H	V	E	K	G	160
481	GTA	CGT	TGT	ACC	GTT	GTT	TGT	ATG	CCA	GTA	CCG	ATT	GAA	GAA	GCC	TCC	528
161	V	R	C	T	V	V	C	M	P	V	P	I	E	E	A	S	176
529	GCA	TTT	GGC	GTT	ATG	GCG	GTT	GAT	GAG	AAC	GAT	AAA	ACT	ATC	GAA	TTC	576
177	A	F	G	V	M	A	V	D	E	N	D	K	T	I	E	F	192
577	GTG	GAA	AAA	CCT	GCT	AAC	CCG	CCG	TCA	ATG	CCG	AAC	GAT	CCG	AGC	AAA	624
193	V	E	K	P	A	N	P	P	S	M	P	N	D	P	S	K	208
625	TCT	CTG	GCG	AGT	ATG	GGT	ATC	TAC	GTC	TTT	GAC	GCC	GAC	TAT	CTG	TAT	672
209	S	L	A	S	M	G	I	Y	V	F	D	A	D	Y	L	Y	224

Fig 46 continued

673	GAA	CTG	CTG	GAA	GAA	GAC	GAT	CGC	GAT	GAG	AAC	TCC	AGC	CAC	GAC	TTT	720
225	E	L	L	E	E	D	D	R	D	E	N	S	S	H	D	F	240
721	GGC	AAA	GAT	TTG	ATT	CCC	AAG	ATC	ACC	GAA	GCC	GGT	CTG	GCC	TAT	GCG	768
241	G	K	D	L	I	P	K	I	T	E	A	G	L	A	Y	A	256
769	CAC	CCG	TTC	CCG	CTC	TCT	TGC	GTA	CAA	TCC	GAC	CCG	GAT	GCC	GAG	CCG	816
257	H	P	F	P	L	S	C	V	Q	S	D	P	D	A	E	P	272
817	TAC	TGG	CGC	GAT	GTG	GGT	ACG	CTG	GAA	GCT	TAC	TGG	AAA	GCG	AAC	CTC	864
273	Y	W	R	D	V	G	T	L	E	A	Y	W	K	A	N	L	288
865	GAT	CTG	GCC	TCT	GTG	GTG	GAC	AAA	CTG	GAT	ATG	TAC	GAT	CGC	AAT	TGG	912
289	D	L	A	S	V	V	D	K	L	D	M	Y	D	R	N	W	304
913	CCA	ATT	CGC	ACC	TAC	AAT	GAA	TCA	TTA	CCG	CCA	GCG	AAA	TTC	GTG	CAG	960
305	P	I	R	T	Y	N	E	S	L	P	P	A	K	F	V	Q	320
961	GAT	CGC	TCC	GGT	AGC	CAC	GGG	ATG	ACC	CTT	AAC	TCA	CTG	GTT	TCC	GGC	1008
321	D	R	S	G	S	H	G	M	T	L	N	S	L	V	S	G	336
1009	GGT	TGT	GTG	ATC	TCC	GGT	TCG	GTG	GTG	GTG	CAG	TCC	GTT	CTG	TTC	TCG	1056
337	G	C	V	I	S	G	S	V	V	V	Q	S	V	L	F	S	352
1057	CGC	GTT	CGC	GTG	AAT	TCA	TTC	TGC	AAC	ATT	GAT	TCC	GCC	GTA	TTG	TTA	1104
353	R	V	R	V	N	S	F	C	N	I	D	S	A	V	L	L	368
1105	CCG	GAA	GTA	TGG	GTA	GGT	CGC	TCG	TGC	CGT	CTG	CGC	CGC	TGC	GTC	ATC	1152
369	P	E	V	W	V	G	R	S	C	R	L	R	R	C	V	I	384
1153	GAT	CGT	GCT	TGT	GTT	ATT	CCG	GAA	GGC	ATG	GTG	ATT	GGT	GAA	AAC	GCA	1200
385	D	R	A	C	V	I	P	E	G	M	V	I	G	E	N	A	400
1201	GAG	GAA	GAT	GCA	CGT	CGT	TTC	TAT	CGT	TCA	GAA	GAA	GGC	ATC	GTG	CTG	1248
401	E	E	D	A	R	R	F	Y	R	S	E	E	G	I	V	L	416
1249	GTA	ACG	CGC	GAA	ATG	CTA	CGG	AAG	TTA	GGG	CAT	AAA	CAG	GAG	CGA	TAA	1296
417	V	T	R	E	M	L	R	K	L	G	H	K	Q	E	R	*	432
1297	TGC	AGG	TTT	TAC	ATG	TAT	GTT	CAG	AGA	TGT	TT						1328
433	C	R	F	Y	M	Y	V	Q	R	C							442

Fig. 47

DNASIS ***** DNA TRANSLATION LIST ***** DATE 04-03-97

*** INPUT INFORMATION ***

FILE NAME : ECGLGC.DNA SEQUENCE : NORMAL 1328 BP

CODON TABLE : UNIV.TCN

SEQUENCE REGION : 1 - 1328

TRANSLATION REGION : 1 - 1326

*** DNA TRANSLATION ***

1	ATG	GTT	AGT	TTA	GAG	AAG	AAC	GAT	CAC	TTA	ATG	TTG	GCG	CGC	CAG	CTG	48
1	M	V	S	L	E	K	N	D	H	L	M	L	A	R	Q	L	16
49	CCA	TTG	AAA	TCT	GTT	GCC	CTG	ATA	CTG	GCG	GGA	GGA	CGT	GGT	ACC	CGC	96
17	P	L	K	S	V	A	L	I	L	A	G	G	R	G	T	R	32
97	CTG	AAG	GAT	TTA	ACC	AAT	AAG	CGA	GCA	AAA	CCG	GCC	GTA	CAC	TTC	GGC	144
33	L	K	D	L	T	N	K	R	A	K	P	A	V	H	F	G	48
145	GGT	AAG	TTC	CGC	ATT	ATC	GAC	TTT	GCG	CTG	TCT	AAC	TGC	ATC	AAC	TCC	192
49	G	K	F	R	I	I	D	F	A	L	S	N	C	I	N	S	64
193	GGG	ATC	CGT	CGT	ATG	GGC	GTG	ATC	ACC	CAG	TAC	CAG	TCC	CAC	ACT	CTG	240
65	G	I	R	R	M	G	V	I	T	Q	Y	Q	S	H	T	L	80
241	GTG	CAG	CAC	ATT	CAG	CGC	GGC	TGG	TCA	TTC	TTC	AAT	GAA	GAA	ATG	AAC	288
81	V	Q	H	I	Q	R	G	W	S	F	F	N	E	E	M	N	96
289	GAG	TTT	GTC	GAT	CTG	CTG	CCA	GCA	CAG	CAG	AGA	ATG	AAA	GGG	GAA	AAC	336
97	E	F	V	D	L	L	P	A	Q	Q	R	M	K	G	E	N	112
337	TGG	TAT	CGC	GGC	ACC	GCA	GAT	GCG	GTC	ACC	CAA	AAC	CTC	GAC	ATT	ATC	384
113	W	Y	R	G	T	A	D	A	V	T	Q	N	L	D	I	I	128
385	CGT	CGT	TAT	AAA	GCG	GAA	TAC	GTG	GTG	ATC	CTG	GCG	GGC	GAC	CAT	ATC	432
129	R	R	Y	K	A	E	Y	V	V	I	L	A	G	D	H	I	144
433	TAC	AAG	CAA	GAC	TAC	TCG	CGT	ATG	CTT	ATC	GAT	CAC	GTC	GAA	AAA	GGT	480
145	Y	K	Q	D	Y	S	R	M	L	I	D	H	V	E	K	G	160
481	GTA	CGT	TGT	ACC	GTT	GTT	TGT	ATG	CCA	GTA	CCG	ATT	GAA	GAA	GCC	TCC	528
161	V	R	C	T	V	V	C	M	P	V	P	I	E	E	A	S	176
529	GCA	TTT	GGC	GTT	ATG	GCG	GTT	GAT	GAG	AAC	GAT	AAA	ACT	ATC	GAA	TTC	576
177	A	F	G	V	M	A	V	D	E	N	D	K	T	I	E	F	192
577	GTG	GAA	AAA	CCT	GCT	AAC	CCG	CCG	TCA	ATG	CCG	AAC	GAT	CCG	AGC	AAA	624
193	V	E	K	P	A	N	P	P	S	M	P	N	D	P	S	K	208
625	TCT	CTG	GCG	AGT	ATG	GGT	ATC	TAC	GTC	TTT	GAC	GCC	GAC	TAT	CTG	TAT	672
209	S	L	A	S	M	G	I	Y	V	F	D	A	D	Y	L	Y	224

57/90

Fig. 47 continued

673	GAA	CTG	CTG	GAA	GAA	GAC	GAT	CGC	GAT	GAG	AAC	TCC	AGC	CAC	GAC	TTT	720
225	E	L	L	E	E	D	D	R	D	E	N	S	S	H	D	F	240
721	GGC	AAA	GAT	TTG	ATT	CCC	AAG	ATC	ACC	GAA	GCC	GGT	CTG	GCC	TAT	GCG	768
241	G	K	D	L	I	P	K	I	T	E	A	G	L	A	Y	A	256
769	CAC	CCG	TTC	CCG	CTC	TCT	TGC	GTA	CAA	TCC	GAC	CCG	GAT	GCC	GAG	CCG	816
257	H	P	F	P	L	S	C	V	Q	S	D	P	D	A	E	P	272
817	TAC	TGG	CGC	GAT	GTG	GGT	ACG	CTG	GAA	GCT	TAC	TGG	AAA	GCG	AAC	CTC	864
273	Y	W	R	D	V	G	T	L	E	A	Y	W	K	A	N	L	288
865	GAT	CTG	GCC	TCT	GTG	GTG	CCG	AAA	CTG	GAT	ATG	TAC	GAT	CGC	AAT	TGG	912
289	D	L	A	S	V	V	P	K	L	D	M	Y	D	R	N	W	304
913	CCA	ATT	CGC	ACC	TAC	AAT	GAA	TCA	TTA	CCG	CCA	GCG	AAA	TTC	GTG	CAG	960
305	P	I	R	T	Y	N	E	S	L	P	P	A	K	F	V	Q	320
961	GAT	CGC	TCC	GGT	AGC	CAC	GGG	ATG	ACC	CTT	AAC	TCA	CTG	GTT	TCC	GGC	1008
321	D	R	S	G	S	H	G	M	T	L	N	S	L	V	S	G	336
1009	GGT	TGT	GTG	ATC	TCC	GGT	TCG	GTG	GTG	GTG	CAG	TCC	GTT	CTG	TTC	TCG	1056
337	G	C	V	I	S	G	S	V	V	V	Q	S	V	L	F	S	352
1057	CGC	GTT	CGC	GTG	AAT	TCA	TTC	TGC	AAC	ATT	GAT	TCC	GCC	GTA	TTG	TTA	1104
353	R	V	R	V	N	S	F	C	N	I	D	S	A	V	L	L	368
1105	CCG	GAA	GTA	TGG	GTA	GGT	CGC	TCG	TGC	CGT	CTG	CGC	CGC	TGC	GTC	ATC	1152
369	P	E	V	W	V	G	R	S	C	R	L	R	R	C	V	I	384
1153	GAT	CGT	GCT	TGT	GTT	ATT	CCG	GAA	GGC	ATG	GTG	ATT	GGT	GAA	AAC	GCA	1200
385	D	R	A	C	V	I	P	E	G	M	V	I	G	E	N	A	400
1201	GAG	GAA	GAT	GCA	CGT	CGT	TTC	TAT	CGT	TCA	GAA	GAA	GGC	ATC	GTG	CTG	1248
401	E	E	D	A	R	R	F	Y	R	S	E	E	G	I	V	L	416
1249	GTA	ACG	CGC	GAA	ATG	CTA	CGG	AAG	TTA	GGG	CAT	AAA	CAG	GAG	CGA	TAA	1296
417	V	T	R	E	M	L	R	K	L	G	H	K	Q	E	R	*	432
1297	TGC	AGG	TTT	TAC	ATG	TAT	GTT	CAG	AGA	TGT	TT						1328
433	C	R	F	Y	M	Y	V	Q	R	C							442

DNASIS ***** DNA TRANSLATION LIST ***** DATE 04-03-97

Fig. 48

*** INPUT INFORMATION ***

FILE NAME : ECGLGCWT.SEQ SEQUENCE : NORMAL 1328 BP

CODON TABLE : UNIV.TCN

SEQUENCE REGION : 1 - 1328

TRANSLATION REGION : 1 - 1326

*** DNA TRANSLATION ***

1	ATG	GTT	AGT	TTA	GAG	AAG	AAC	GAT	CAC	TTA	ATG	TTG	GCG	CGC	CAG	CTG	48
1	M	V	S	L	E	K	N	D	H	L	M	L	A	R	Q	L	16
49	CCA	TTG	AAA	TCT	GTT	GCC	CTG	ATA	CTG	GCG	GGA	GGA	CGT	GGT	ACC	CGC	96
17	P	L	K	S	V	A	L	I	L	A	G	G	R	G	T	R	32
97	CTG	AAG	GAT	TTA	ACC	AAT	AAG	CGA	GCA	AAA	CCG	GCC	GTA	CAC	TTC	GGC	144
33	L	K	D	L	T	N	K	R	A	K	P	A	V	H	F	G	48
145	GGT	AAG	TTC	CGC	ATT	ATC	GAC	TTT	GCG	CTG	TCT	AAC	TGC	ATC	AAC	TCC	192
49	G	K	F	R	I	I	D	F	A	L	S	N	C	I	N	S	64
193	GGG	ATC	CGT	CGT	ATG	GGC	GTG	ATC	ACC	CAG	TAC	CAG	TCC	CAC	ACT	CTG	240
65	G	I	R	R	M	G	V	I	T	Q	Y	Q	S	H	T	L	80
241	GTG	CAG	CAC	ATT	CAG	CGC	GGC	TGG	TCA	TTC	TTC	AAT	GAA	GAA	ATG	AAC	288
81	V	Q	H	I	Q	R	G	W	S	F	F	N	E	E	M	N	96
289	GAG	TTT	GTC	GAT	CTG	CTG	CCA	GCA	CAG	CAG	AGA	ATG	AAA	GGG	GAA	AAC	336
97	E	F	V	D	L	L	P	A	Q	Q	R	M	K	G	E	N	112
337	TGG	TAT	CGC	GGC	ACC	GCA	GAT	GCG	GTC	ACC	CAA	AAC	CTC	GAC	ATT	ATC	384
113	W	Y	R	G	T	A	D	A	V	T	Q	N	L	D	I	I	128
385	CGT	CGT	TAT	AAA	GCG	GAA	TAC	GTG	GTG	ATC	CTG	GCG	GGC	GAC	CAT	ATC	432
129	R	R	Y	K	A	E	Y	V	V	I	L	A	G	D	H	I	144
433	TAC	AAG	CAA	GAC	TAC	TCG	CGT	ATG	CTT	ATC	GAT	CAC	GTC	GAA	AAA	GGT	480
145	Y	K	Q	D	Y	S	R	M	L	I	D	H	V	E	K	G	160
481	GTA	CGT	TGT	ACC	GTT	GTT	TGT	ATG	CCA	GTA	CCG	ATT	GAA	GAA	GCC	TCC	528
161	V	R	C	T	V	V	C	M	P	V	P	I	E	E	A	S	176
529	GCA	TTT	GGC	GTT	ATG	GCG	GTT	GAT	GAG	AAC	GAT	AAA	ACT	ATC	GAA	TTC	576
177	A	F	G	V	M	A	V	D	E	N	D	K	T	I	E	F	192
577	GTG	GAA	AAA	CCT	GCT	AAC	CCG	CCG	TCA	ATG	CCG	AAC	GAT	CCG	AGC	AAA	624
193	V	E	K	P	A	N	P	P	S	M	P	N	D	P	S	K	208
625	TCT	CTG	GCG	AGT	ATG	GGT	ATC	TAC	GTC	TTT	GAC	GCC	GAC	TAT	CTG	TAT	672
209	S	L	A	S	M	G	I	Y	V	F	D	A	D	Y	L	Y	224

Fig. 48 Continued

673	GAA	CTG	CTG	GAA	GAA	GAC	GAT	CGC	GAT	GAG	AAC	TCC	AGC	CAC	GAC	TTT	720
225	E	L	L	E	E	D	D	R	D	E	N	S	S	H	D	F	240
721	GGC	AAA	GAT	TTG	ATT	CCC	AAG	ATC	ACC	GAA	GCC	GGT	CTG	GCC	TAT	GCG	768
241	G	K	D	L	I	P	K	I	T	E	A	G	L	A	Y	A	256
769	CAC	CCG	TTC	CCG	CTC	TCT	TGC	GTA	CAA	TCC	GAC	CCG	GAT	GCC	GAG	CCG	816
257	H	P	F	P	L	S	C	V	Q	S	D	P	D	A	E	P	272
817	TAC	TGG	CGC	GAT	GTG	GGT	ACG	CTG	GAA	GCT	TAC	TGG	AAA	GCG	AAC	CTC	864
273	Y	W	R	D	V	G	T	L	E	A	Y	W	K	A	N	L	288
865	GAT	CTG	GCC	TCT	GTG	GTG	CCG	GAA	CTG	GAT	ATG	TAC	GAT	CGC	AAT	TGG	912
289	D	L	A	S	V	V	P	E	L	D	M	Y	D	R	N	W	304
913	CCA	ATT	CGC	ACC	TAC	AAT	GAA	TCA	TTA	CCG	CCA	GCG	AAA	TTC	GTG	CAG	960
305	P	I	R	T	Y	N	E	S	L	P	P	A	K	F	V	Q	320
961	GAT	CGC	TCC	GGT	AGC	CAC	GGG	ATG	ACC	CTT	AAC	TCA	CTG	GTT	TCC	GGC	1008
321	D	R	S	G	S	H	G	M	T	L	N	S	L	V	S	G	336
1009	GGT	TGT	GTG	ATC	TCC	GGT	TCG	GTG	GTG	GTG	CAG	TCC	GTT	CTG	TTC	TCG	1056
337	G	C	V	I	S	G	S	V	V	V	Q	S	V	L	F	S	352
1057	CGC	GTT	CGC	GTG	AAT	TCA	TTC	TGC	AAC	ATT	GAT	TCC	GCC	GTA	TTG	TTA	1104
353	R	V	R	V	N	S	F	C	N	I	D	S	A	V	L	L	368
1105	CCG	GAA	GTA	TGG	GTA	GGT	CGC	TCG	TGC	CGT	CTG	CGC	CGC	TGC	GTC	ATC	1152
369	P	E	V	W	V	G	R	S	C	R	L	R	R	C	V	I	384
1153	GAT	CGT	GCT	TGT	GTT	ATT	CCG	GAA	GGC	ATG	GTG	ATT	GGT	GAA	AAC	GCA	1200
385	D	R	A	C	V	I	P	E	G	M	V	I	G	E	N	A	400
1201	GAG	GAA	GAT	GCA	CGT	CGT	TTC	TAT	CGT	TCA	GAA	GAA	GGC	ATC	GTG	CTG	1248
401	E	E	D	A	R	R	F	Y	R	S	E	E	G	I	V	L	416
1249	GTA	ACG	CGC	GAA	ATG	CTA	CGG	AAG	TTA	GGG	CAT	AAA	CAG	GAG	CGA	TAA	1296
417	V	T	R	E	M	L	R	K	L	G	H	K	Q	E	R	*	432
1297	TGC	AGG	TTT	TAC	ATG	TAT	GTT	CAG	AGA	TGT	TT						1328
433	C	R	F	Y	M	Y	V	Q	R	C							442

Fig 49

```
(Linear) MAP of: Waxy0.Seq  check: 2247  from: 1  to: 1917

With 189 enzymes: *

                           June 10, 1993  17:59  ..

     F          H          F                 B            EH           A
     n C       C aH        n  C              s C B C CBca  B SB     S f
     Au v   ME vHeaM       u Av         B    sMaHsHaHvsoeMNsHcsTMNcTlM
     c4 i   wa iaIes       4 li         b    HwcgrhchiaRIsaagrmhscrhIl
     iH J   oe JeIIc       H uJ         v    Io8aFa8aJJIIpeHaFFapiFaIu
     II I   II IIIII       I II         I    IIIIIIIIIIIIIIIIIIIIIIIII
               /// /          /                / /  //// // / /// /
    ATGgcggctctggccacgtcgcagctcgtcgcaacgcgcgccggcctgggcgtcccggac
  1 ---------+---------+---------+---------+---------+---------+ 60
    TACcgccgagaccggtgcagcgtcgagcagcgttgcgcgcggccggacccgcagggcctg

    M  A  A  L  A  T  S  Q  L  V  A  T  R  A  G  L  G  V  P  D  -

                                      E           E  SmaI
                                      c           c     B
                           FF F       o    B HN   o     s
           M        B      Bnn nH     ONCAs as   SONC  BB1Ba     F  F
    T      Ha    AE AsADBsuuAuaHHNM1lvluDepHBallvAAas2seBMNNAuMAul
    h      ge    cc cacsaa44c4ehhan0aiw3dIBgsc0aivpna8aIsscc4wc4a
    a      aI    ii iJianHHHiHIaarl9IJN6eIIalI9IJaaIJ6HIlpiiiHoiHI
    I      II    II IIIIIIIIIIIIIIIVIIIIIIIIIIVIIIIIIIIIIIIIIIIIIV
           /        / /  // /// /  /    ///  /      /// / ///  /  /
    gcgtccacgttccgccgcggcgccgcgcagggcctgagggggcccgggcgtcggcggcg
 61 ---------+---------+---------+---------+---------+---------+ 120
    cgcaggtgcaaggcggcgccgcggcgcgtcccggactcccccgggcccgcagccgccgc

    A  S  T  F  R  R  G  A  A  Q  G  L  R  G  A  R  A  S  A  A  -

                 B
                 p
      SSS        u        N  S    B B B    F     E          F     B
       aaaSS     1    C   lA a    s sCsCC  n B   BcH     NN nS  C sC
    AEuuuccSS1DTT Ma AavNuS  s sasaaHAuBsHHsoaHBHMMN1lMucBTaMAsa
    cc999rrmr0dhh wc cIas9p  H HcHcchc4aahhaRehahwwaaaw4rshcwsHc
    ii666FFaf2eaa o8 iIIp6h  I I8I88aiHnHaaJIIanaoorIIoHFla8ocI8
    IIIIIIIIIIIII II IIIIII  I IIIIIIIIIIIIIIIIIIIIIVVIIIIIIIIIII
     /////// ///       / ///     / / /    // /// //////  / /   /
    gcggacacgctcagcatgcggaccagcgcgcgcgcggcgcccaggcaccagcagcaggcg
121 ---------+---------+---------+---------+---------+---------+ 180
    cgcctgtgcgagtcgtacgcctggtcgcgcgcgcgccgcgggtccgtggtcgtcgtccgc

    A  D  T  L  S  M  R  T  S  A  R  A  A  P  R  H  Q  Q  Q  A  -

           F  N                                         N
     B  B  n  sS N  B                 C     BHC        MM1
    FsABAsDBuHHMpaT1TTsTT        BH   a     saaHMN    Bbaa
    apcbcasg4hhwBchahhchh        ch   c     rechsa    boeI
    uMiviJalHaaoIIaIaaGaa        ga   8     FI8ape    sIII
    IIIIIIIIIIIIIIIVIIIII        II   I     IIIIII    IIII
    / / // /////// // //  ct ggtaccgta tg     ///      / /
    cgccgcgggggcaggttcccgtcgctcgtcgtgtgcgccagcgcggcatgaacgtcgtc
181 ---------+---------+---------+---------+---------+---------+ 240
    gcggcgcccccgtccaagggcagcgagcagcacacgcggtcgcggccgtacttgcagcag

    R  R  G  G  R  F  P  S  L  V  V  C  A  S  A  G  M  N  V  V  -
                                              NdeI
                                              CATATG
                          216                 TAC -
```

Fig 49 continued

```
                                            F    H                  F
  BB H   N    B  B H N           B        n CBa      BM AB          n
  BcsBaHNl BBBsMHsDaNl          sMM Au vseM       saMas          Au
  aeacehaa gcaawhaseaa          rsw c4 iaIw       aenta          c4
  nfHgIarI lcnHoaJaIrI          Fpo iH JJIo       HIlIJ          iH
  IIIIIIIV IIIIIIIIIIV          III II IIII       IIIII          II
   // ///     /  ////             /     /          /
  ttcgtcggcgccgagatggcgccgtggagcaagaccggcggcctcggcgacgtcctcggc
241 ---------+---------+---------+---------+---------+---------+ 300
  aagcagccgcggctctaccgcggcacctcgttctggccgccggagccgctgcaggagccg

a  F  V  G  A  E  M  A  P  W  S  K  T  G  G  L  G  D  V  L  G  -

                                    B                           P
                                    s                           f
   H     F             FHH          Np           N              l
  CaC    n BC CB G GC naa       B  llN           l              l
  veaM AuBsaEvsDdEdvAueeMMBNsBNa2lST    B  a          BE        l
  iIcn c4srcaiasiaiic4IIswsacacI8ath    s  I          ss A      0
  JI8l iHlF8eJJaIeIJiHIIpoleGnoI6Iya    l  I          mp c      8
  IIII IIIIIIIIIIIIIIIIIIIIIIIIIIVII    I  I          A3 i      I
   / /  / / /  // /  ///// /  // ///                  II I
                                                        /
  ggcctgccgccggccatggccgcgaacgggcaccgtgtcatggtcgtctctccccgctac
301 ---------+---------+---------+---------+---------+---------+ 360
  ccggacggcggccggtaccggcgcttgcccgtggcacagtaccagcagagaggggcgatg

a  G  L  P  P  A  M  A  A  N  G  H  R  V  M  V  V  S  P  R  Y  -

                  E
               B cB S  B                     S
  F B   R B    s osHc  sH     D              a       BE         BBE
  a s   s s    a Ragr  mg     r              u D     ss B       sss
  u r   a l    H IJaF  Fa     d              3 p     mp c       cmp
  I I   I I    I IIII  II     I              A n     A3 c       GA3
                                             I I     II I       III
                  /  /                                /          //
  gaccagtacaaggacgcctgggacaccagcgtcgtgtccgagatcaagatgggagacggg
361 ---------+---------+---------+---------+---------+---------+ 420
  ctggtcatgttcctgcggaccctgtggtcgcagcacaggctctagttctaccctctgccc

a  D  Q  Y  K  D  A  W  D  T  S  V  V  S  E  I  K  M  G  D  G  -
```

411 →

```
                                                 S              H
        M                                        Aa             i
  R     b                        M  AHT          vu A T         n
  s     o                        w  chh          a9 c h         c
  a     I                        o  iaa          I6 i a         I
  I     I                        I  III          II I I         I
                                     //           /
  tacgagacggtcaggttcttccactgctacaagcgcggagtggaccgcgtgttcgttgac
421 ---------+---------+---------+---------+---------+---------+ 480
  atgctctgccagtccaagaaggtgacgatgttcgcgcctcacctggcgcacaagcaactg

a  Y  E  T  V  R  F  F  H  C  Y  K  R  G  V  D  R  V  F  V  D  -

        E
        c S   T                                   S     S H
        oMc   aB             B     B         BB a T    BaCaM
        Rnr   qs        M    Bs    s         gsDu a    suveb
        IlF   Il        n    pa    e         ltp3 q    c9iIo
        III   II        l    mJ    R         IYnA I    G6JII
                        I    II    I         IIII I    IIIII
          /                                    / /      / //
  cacccactgttcctggagagggtttggggaaagaccgaggagaagatctacgggcctgtc
481 ---------+---------+---------+---------+---------+---------+ 540
  gtgggtgacaaggacctctcccaaacccctttctggctcctcttctagatgcccggacag

a  H  P  L  F  L  E  R  V  W  G  K  T  E  E  K  I  Y  G  P  V  -
```

Fig 49 continued

```
                                                                B
                                                                s
                                      F HN                E     p
                      B              Cn gsP     C C      cBS    1H
B          S B        s              AvuAipv    v a   M  osc    2g
s          f b        m              li4cEBu    i c   w  Rar    8i
l          c v        F              uJHiIII    J 8   o  IJF    6A
I          I I        I              IIIIIII    I I   I  III    II
                                      // ///                     /
    gctggaacggactacagggacaaccagctgcggttcagcctgctatgccagggagcactt
541 ---------+---------+---------+---------+---------+---------+ 600
    cgaccttgcctgatgtccctgttggtcgacgccaagtcggacgatacggtccctcgtgaa

a   A  G  T  D  Y  R  D  N  Q  L  R  F  S  L  L  C  Q  G  A  L  -

                   SB                                 S
    C B      BB Nap   C                        BB Aa         B
M   AvAsS    asDluuD Av          M             ssMvu  M      s
w   lilat    mtpa3ld li          n             apsa9  n      c
o   uJwJy    HYnIA0e wJ          l             WEpI6  l      G
I   IIIII    IIIVIII II          I             IIIII  I      I
     /  /   ~/ /// /                            // /
    gaagctccaaggatcctgagcctcaacaacaacccatacttctccggaccatacgggagg
601 ---------+---------+---------+---------+---------+---------+ 660
    cttcgaggttcctaggactcggagttgttgttgggtatgaagaggcctggtatgccctcc

a   E  A  P  R  I  L  S  L  N  N  N  P  Y  F  S  G  P  Y  G  R  -

                                      S HB
BBM A             C              B   aCac
ssa a             v          B   sM  uvee   B    M
mae t             i          s   rs  9iI8   s    n
FHI I             R          r   Fp  6JI3   i    l
III I             I          I   II  IIII   I    I
  /                                  /
    gacgtcgtgttcgtctgcaacgactggcacaccggccctctctcgtgctacctcaagagc
661 ---------+---------+---------+---------+---------+---------+ 720
    ctgcagcacaagcagacgttgctgaccgtgtggccgggagagagcacgatggagttctcg

a   D  V  V  F  V  C  N  D  W  H  T  G  P  L  S  C  Y  L  K  S  -

              B B                S.B B                C          S
M      B      s sD        S      f s c   F   HAM      v          f
n      s      m as        f      a m e   o   gcw      i          a
l      r      F Ja        c      N F f   k   aio      R          N
I      I      I II        I      I I I   I   III      I          I
                /                              /
    aactaccagtcccacggcatctacagggacgcaaagaccgctttctgcatccacaacatc
721 ---------+---------+---------+---------+---------+---------+ 780
    ttgatggtcagggtgccgtagatgtccctgcgtttctggcgaaagacgtaggtgttgtag

a   N  Y  Q  S  H  G  I  Y  R  D  A  K  T  A  F  C  I  H  N  I  -

     E   SH  H
     cBS agBCa                      S   C               S   H
     osc uisveM                    MNc Av            MMNc  MiT
     Rar 9EriIs                    scr li            smcr  nnf
     IJF 6IFJIp                    piF uJ            peiF  lfi
     III IIIIII                    III II            IIII  III
           ///                      //   /             //    /
    tcctaccagggccggttcgccttctccgactacccggagctgaacctcccggagagattc
781 ---------+---------+---------+---------+---------+---------+ 840
    aggatggtcccggccaagcggaagaggctgatgggcctcgacttggagggcctctctaag

a   S  Y  Q  G  R  F  A  F  S  D  Y  P  E  L  N  L  P  E  R  F  -
```

63/90

Fig 49 continued

```
                                      P
                                      f
                                      l
                                  C   l     C BBB      H    S
              T        T          v   l     v scsD B   Ca   a
              a        a          i   0     i aecs s   veM  u D
              q        q          J   8     J JfGa l   iIs  3 p
              I        I          I   I     I IIII I   JIp  A n
                                                       III  I I
                                                //     //
     aagtcgtccttcgatttcatcgacggctacgagaagcccgtggaaggccggaagatcaac
 841 ---------+---------+---------+---------+---------+---------+ 900
     ttcagcaggaagctaaagtagctgccgatgctcttcgggcaccttccggccttctagttg

a    K  S  S  F  D  F  I  D  G  Y  E  K  P  V  E  G  R  K  I  N  -

                                        E
                                    T   c
         H         S      H         t   o S
   M    Ca  BB     Na S Ca          h AONPa          C        B
   bB   AveMNasDFMAluAcTveXM H1 v1lpu         Mv     M   s
   os   liIscmtponva3lraiIhn pl a0au9         ni     n   a
   Ir   wJIpiHYnklaIAwFqJIol hl I9IM6         lJ     l   J
   II   IIIIIIIIIIVIIIIIIII  II IIVII         II     I   I
          /// / // // // //     ////
     tggatgaaggccgggatcctcgaggccgacagggtcctcaccgtcagcccctactacgcc
 901 ---------+---------+---------+---------+---------+---------+ 960
     acctacttccggccctaggagctccggctgtcccaggagtggcagtcggggatgatgcgg

a    W  M  K  A  G  I  L  E  A  D  R  V  L  T  V  S  P  Y  Y  A  -

          B                                  B
          s                                  s
          p             E        F           p            N
   B    CB1H            cBS S   ACn   C    CB1H       l       B
   s    Ava2gS  M B     osc f   lvu   a   Ava2gST   H a H   HsBM
   e    lin8is  s b     Rar a   wi4   c   lin8isa   p I h   prgs
   R    uJI6At  p v     IJF N   NJH   8   uJI6Atq   h I a   hFlp
   I    IIIIII  I I     III I   III   I   IIIIIII   I I I   IIII
         / ///                   //        / ////              /
     gaggagctcatctccggcatcgccaggggctgcgagctcgacaacatcatgcgcctcacc
 961 ---------+---------+---------+---------+---------+---------+ 1020
     ctcctcgagtagaggccgtagcggtccccgacgctcgagctgttgtagtacgcggagtgg

a    E  E  L  I  S  G  I  A  R  G  C  E  L  D  N  I  M  R  L  T  -

                                                E
                                                c
                      H        N               o  S
          B   S       i S      l BM A   B     ABONNPa   B B
   M M    sMM f       n f      a sa a   c     vs1llpu B c s
   w n    rsw a       c a      I ae t   e     am0aau9 s e m
   o l    Fpo N       I N      I HI I   f     IF9IIM6 l f F
   I I    III I       I I      I II I   I     IIIVVII I I I
   /       /                      /            /////
     ggcatcaccggcatcgtcaacggcatggacgtcagcgagtgggaccccagcagggacaag
1021 ---------+---------+---------+---------+---------+---------+ 1080
     ccgtagtggccgtagcagttgccgtacctgcagtcgctcaccctggggtcgtccctgttc

a    G  I  T  G  I  V  N  G  M  D  V  S  E  W  D  P  S  R  D  K  -
```

4

Fig 49 continued

```
                            A    H       H     H
                      B    fM    i    CBB Ga   CB aB    H
R     M               Rc   la   SAnT EEvssDdeBMMvsHecSS HaBM
s     s               se   Ie   acca aaiaisiIgnwiaaIeft hebn
a     l               af   II   lcIq egJJEaIIlloJJeIfiy aIvl
I     I               II   II   IIII IIIIIIIIIIIIIIIIII IIII
                            /     /  /  //// //  // //
     tacatcgccgtgaagtacgacgtgtcgacggccgtggaggccaaggcgctgaacaaggag
1081 ---------+---------+---------+---------+---------+---------+ 1140
     atgtagcggcacttcatgctgcacagctgccggcacctccggttccgcgacttgttcctc

     Y  I  A  V  K  Y  D  V  S  T  A  V  E  A  K  A  L  N  K  E  -

                            B
                            s
   F                        p     S           N
   nHC C                  CNBl  S AaB         s
  HuavMaMAEPS             vla2FMNc vusM     A p  B F
  h4eincwccsf             ian8oscr a9as     c B  s a
  aHIRl8oiitc             JII6kpiF I6Wp     i I  l u
  IIIIIIIIIII             IVIIIIII IIII     I I  I I
    / / /  //              / ///  /
     gcgctgcaggcggaggtcgggctcccggtggaccggaacatcccgctggtggcgttcatc
1141 ---------+---------+---------+---------+---------+---------+ 1200
     cgcgacgtccgcctccagcccgagggccacctggccttgtagggcgaccaccgcaagtag

     A  L  Q  A  E  V  G  L  P  V  D  R  N  I  P  L  V  A  F  I  -

                         EE  B
                         cc  s
                         oo H p      SN     FF  H  S F      N
   C C                   OOCa BlMBM ANNal  C Bnn Ga  a n  C  l
   aEvS    M             llveAa2bsaFalluaAEEvAsuuBdeNAu u AvF a
   caia    w             OOiIpn8oaeotaa9Icaaici44ciIoc9 4 lia I
   8rJp    o             99JIaI6IHIkIII6IiegJiEHHcIIti6 H uJu I
   IIII    I             IIIIIIIIIIIVVIIIIIIIIIIIIIIIII I III I
     /                      / /// /  ///  /  /// /// /    /
     ggcaggctggaagagcagaagggccccgacgtcatggcggccgccatcccgcagctcatg
1201 ---------+---------+---------+---------+---------+---------+ 1260
     ccgtccgaccttctcgtcttcccggggctgcagtaccgccggcggtagggcgtcgagtac

     G  R  L  E  E  Q  K  G  P  D  V  M  A  A  A  I  P  Q  L  M  -

                                  B
                                  s
                    S             p
              M  C a              lB              M
M BMB         a  v u D            B2s      X   T  b H
s bnc         e  i 3 p            s8c      m   a  o h
l vlc         I  R A n            g6G      n   q  I a
I III         I  I I I            III      I   I  I I
  /                               /
     gagatggtggaggacgtgcagatcgttctgctgggcacgggcaagaagaagttcgagcgc
1261 ---------+---------+---------+---------+---------+---------+ 1320
     ctctaccacctcctgcacgtctagcaagacgacccgtgcccgttcttcttcaagctcgcg

     E  M  V  E  D  V  Q  I  V  L  L  G  T  G  K  K  K  F  E  R  -
```

65/90

Fig 49 continued

```
      N       N
    C l B     l     BH   B             E               B
    a aMsNS  Ma    Hsa   s           BcBS              s C  B
    c Ispsp  nI    hae   e           socc   B        Ms aHHsDT
    8 IlHph  lI    aJI   R          .aRer   s        wH chhash
    I IIIII  II    III   I           JIfF   l        oI 8aaJaa
                                     IIII   I        II IIIIII
       //    /     /                  / /                /  //
     atgctcatgagcgccgaggagaagttcccaggcaaggtgcgcgccgtggtcaagttcaac
1321 ---------+---------+---------+---------+---------+---------+ 1380
     tacgagtactcgcggctcctcttcaagggtccgttccacgcgcggcaccagttcaagttg

a    M  L  M  S  A  E  E  K  F  P  G  K  V  R  A  V  V  K  F  N  -

                                                 B
                                                 s
     F                             H        N    p    M T        F
     n     HC                  P GBCC BBaH   M   l N1CH a s    C n
     AuT   HaaM   H            BfEdsvaBcseaHMaNNaHl2ag e p    vAu     T
     c4h   hecw   h            slairicaeaIehseaaIpa8ci I 4    ic4     a
     iHa   aI8o   a            lMeIFJ8nfHIIapIerIhI68A I 5    JiH     q
     III   IIII   I            IIIIIIIIIIIIIIIIIIIVIII I I    III     I
      /      /                 / / /    // // /// / //          /
     gcggcgctggcgcaccacatcatggccggcgccgacgtgctcgccgtcaccagccgcttc
1381 ---------+---------+---------+---------+---------+---------+ 1440
     cgccgcgaccgcgtggtgtagtaccggccgcggctgcacgagcggcagtggtcggcgaag

a    A  A  L  A  H  H  I  M  A  G  A  D  V  L  A  V  T  S  R  F  -

         B
         s
         p    F H             F   N
       C B1    nCa          S Cn C s P A
     F v a2  ABuve          fAvuBvMpPvMpS                           C
     o i n8  cb4iI          ali4sinBsuwaf         F    H     H  a    HT
     k J I6  ivHJI          NuJHlRlItIoBc         o    g     h  c    hh
     I I II  IIIII          IIIIIIIIIIIII         k    a     a  8    aa
                                                  I    I     I  I    II
          /   // /          // /////  /                               /
     gagccctgcggcctcatccagctgcaggggatgcgatacggaacgccctgcgcctgcgcg
1441 ---------+---------+---------+---------+---------+---------+ 1500
     ctcgggacgccggagtaggtcgacgtcccctacgctatgccttgcgggacgcggacgcgc

a    E  P  C  G  L  I  Q  L  Q  G  M  R  Y  G  T  P  C  A  C  A  -

                           H
       BB   S H            i                                  N  FHS
      AssMPg i          SAnTM   B     T           S    N      1C naa
      garslr n          accal   c     a          MNc   l      HavAueu
      eWFpeA f          lcIqy   c     q          scr   a      gIic4I9
      IIIIII I          IIIII   I     I          piF   I      aIJiHI6
                                                 III   V      IIIIIII
      /////             /                          /          /  ///
     tccaccggtggactcgtcgacaccatcatcgaaggcaagaccgggttccacatgggccgc
1501 ---------+---------+---------+---------+---------+---------+ 1560
     aggtggccacctgagcagctgtggtagtagcttccgttctggcccaaggtgtacccggcg

a    S  T  G  G  L  V  D  T  I  I  E  G  K  T  G  F  H  M  G  R  -

     B       H   M                                       H
     p       i   a   M       NBCC    BM A              C a    B    C
     uDM   SAnMT e   Da      lsvaMAEN sa a           E vHeM   s    v
     ldw   accna I   re      aricscca ae t           a iaIs   r    i
     Oeo   lcIlq I   dI      IFJ8piie HI I           e JeIc   D    R
     III   IIIII I   II      VIIIIIII II I           I IIII   I    I
      //      /               / / /  /                 ///
     ctcagcgtcgactgtaacgtcgtggagccggcggacgtcaagaaggtggccaccacattg
1561 ---------+---------+---------+---------+---------+---------+ 1620
     gagtcgcagctgacattgcagcacctcggccgcctgcagttcttccaccggtggtgtaac

a    L  S  V  D  C  N  V  V  E  P  A  D  V  K  K  V  A  T  T  L  -
```

66/90

Fig 49 continued

```
     F B
     n s C            B           C B C   S        B                     SN
     u s aHHTB  BM s           a sMaMNgSR      s M  B          C  al
     4 H chhhc  bs t           c rscnarus      e n  c        A vH ua
     H I 8aaac  vl X           8 Fp8leAna      R l  c        l ip 3I
     I I IIIII  II I           I IIIIIIII      I I  I        w Rh AI
                                                             I II II
       / /                          ///
     cagcgcgccatcaaggtggtcggcacgccggcgtacgaggagatggtgaggaactgcatg
1621 ---------+---------+---------+---------+---------+---------+ 1680
     gtcgcgcggtagttccaccagccgtgcggccgcatgctcctctaccactccttgacgtac

a    Q  R  A  I  K  V  V  G  T  P  A  Y  E  E  M  V  R  N  C  M  -

                          EE    B                           B
                          cc    s                           p
     E    S    E          oo H  p SS                  F     u
     c S Ba    c S        OOCa BlNaa     P        M   n     l  CB
     Do c suD  oAc        llveAa2luu   BBf  B     a   u    1D  vsA
     pR r t3p  Rlr        00iIpn8a99   bsl  s     e   4    0d  iav
     nI F YAn  IwF        99JIaI6I66   vlM  r     I   H    2e  JJa
     II I III  III        IIIIIIIVII   III  I     I   I    II  III
          /                / /////      //                  /
     atccaggatctctcctggaagggccctgccaagaactgggagaacgtgctgctcagcctc
1681 ---------+---------+---------+---------+---------+---------+ 1740
     taggtcctagagaggaccttcccgggacggttcttgaccctcttgcacgacgagtcggag

a    I  Q  D  L  S  W  K  G  P  A  K  N  W  E  N  V  L  L  S  L  -

              F        E                  S          F
       B C    n S C  CBcS                 B a        nB C    B
       sMaMAuNg a  vsoc    TM       s u D   T HAusMa  sS      X
       rncsc4ar c  iaRr    an       e 3 p   h hc4rwc  at      c
       F18piHeA 8  JJIF    ql       R A n   a aiHBo8  Jy      m
       IIIIIIII I  IIII    II       I I I   I IIIIII  II      I
        / /  //    //                           /       /
     ggggtcgccggcggcgagccaggggtcgaaggcgaggagatcgcgccgctcgccaaggag
1741 ---------+---------+---------+---------+---------+---------+ 1800
     ccccagcggccgccgctcggtccccagcttccgctcctctagcgcggcgagcggttcctc

a    G  V  A  G  G  E  P  G  V  E  G  E  E  I  A  P  L  A  K  E  -

                                          EE    B               S
         FH                               cc    s               s
     M  GC na                    H        oo E  pH     SSS      e
     a  EdvAueTEH                CaCMC    OOCc Bla  NN aaa      8   C
     e  aiic4Ihah                veabvSBllvoAa2eMDllPuuuHS3TMT      v
     I  eIJiHIara                iIcoifg00i5pn8Iwpaas993hf8hwh      i
     I  IIIIIIIII                JI8IRc199J7aI6IonIIt66Aai7aoa      R
                                 IIIIIIIIIIIIIIIIIIVVIIIIIIIIII     I
         /  /// /                / / /    / //// ////// /// /
     aacgtggccgcgccctgaagagttcggcctgcagggcccctgatctcgcgcgtggtgcaa
1801 ---------+---------+---------+---------+---------+---------+ 1860
     ttgcaccggcgcgggacttctcaagccggacgtcccggggactagagcgcgcaccacgtt

a    N  V  A  A  P  *  R  V  R  P  A  G  P  L  I  S  R  V  V  Q  -

       M  B
       b  s
       o  m
       I  F
       I  I
     agatgttgggacatcttcttatatatgctgtttcgtttatgtgatatggacaagt..
1861 ---------+---------+---------+---------+---------+------- 1917
     tctacaaccctgtagaagaatatatacgacaaagcaaatacactatacctgttca..

     R  C  W  D  I  F  L  Y  M  L  F  R  L  C  D  M  D  K  ?  -
```

Fig 50

DNASIS ***** DNA TRANSLATION LIST ***** DATE 04-03-97

*** INPUT INFORMATION ***

FILE NAME : MSS3.SEQ SEQUENCE : NORMAL 2423 BP

CODON TABLE : UNIV.TCN

SEQUENCE REGION : 1 - 2423

TRANSLATION REGION : 1 - 2421

*** DNA TRANSLATION ***

```
  1  ATG CCG GGG GCA ATC TCT TCC TCG TCG TCG GCT TTT CTC CTC CCC GTC   48
  1   M   P   G   A   I   S   S   S   S   S   A   F   L   L   P   V   16

 49  GCG TCC TCC TCG CCG CGG CGC AGG CGG GGC AGT GTG GGT GCT GCT CTG   96
 17   A   S   S   S   P   R   R   R   R   G   S   V   G   A   A   L   32

 97  CGC TCG TAC GGC TAC AGC GGC GCG GAG CTG CGG TTG CAT TGG GCG CGG  144
 33   R   S   Y   G   Y   S   G   A   E   L   R   L   H   W   A   R   48

145  CGG GGC CCG CCT CAG GAT GGA GCG GCG TCG GTA CGC GCC GCA GCG GCA  192
 49   R   G   P   P   Q   D   G   A   A   S   V   R   A   A   A   A   64

193  CCG GCC GGG GGC GAA AGC GAG GAG GCA GCG AAG AGC TCC TCC TCG TCC  240
 65   P   A   G   G   E   S   E   E   A   A   K   S   S   S   S   S   80

241  CAG GCG GGC GCT GTT CAG GGC AGC ACG GCC AAG GCT GTG GAT TCT GCT  288
 81   Q   A   G   A   V   Q   G   S   T   A   K   A   V   D   S   A   96

289  TCA CCT CCC AAT CCT TTG ACA TCT GCT CCG AAG CAA AGT CAG AGC GCT  336
 97   S   P   P   N   P   L   T   S   A   P   K   Q   S   Q   S   A  112

337  GCA ATG CAA AAC GGA ACG AGT GGG GGC AGC AGC GCG AGC ACC GCC GCG  384
113   A   M   Q   N   G   T   S   G   G   S   S   A   S   T   A   A  128

385  CCG GTG TCC GGA CCC AAA GCT GAT CAT CCA TCA GCT CCT GTC ACC AAG  432
129   P   V   S   G   P   K   A   D   H   P   S   A   P   V   T   K  144

433  AGA GAA ATC GAT GCC AGT GCG GTG AAG CCA GAG CCC GCA GGT GAT GAT  480
145   R   E   I   D   A   S   A   V   K   P   E   P   A   G   D   D  160

481  GCT AGA CCG GTG GAA AGC ATA GGC ATC GCT GAA CCG GTG GAT GCT AAG  528
161   A   R   P   V   E   S   I   G   I   A   E   P   V   D   A   K  176

529  GCT GAT GCA GCT CCG GCT ACA GAT GCG GCG GCG AGT GCT CCT TAT GAC  576
177   A   D   A   A   P   A   T   D   A   A   A   S   A   P   Y   D  192

577  AGG GAG GAT AAT GAA CCT GGC CCT TTG GCT GGG CCT AAT GTG ATG AAC  624
193   R   E   D   N   E   P   G   P   L   A   G   P   N   V   M   N  208

625  GTC GTC GTG GTG GCT TCT GAA TGT GCT CCT TTC TGC AAG ACA GGT GGC  672
209   V   V   V   V   A   S   E   C   A   P   F   C   K   T   G   G  224
```

68/90

Fig 50 continued

```
1633  GTG ATG CTG GGC ACC GGG CGG GCC GAC CTG GAG GAC ATG CTG CGG CGG  1680
 545   V   M   L   G   T   G   R   A   D   L   E   D   M   L   R   R   560

1681  TTC GAG TCG GAG CAC AGC GAC AAG GTG CGC GCG TGG GTG GGG TTC TCG  1728
 561   F   E   S   E   H   S   D   K   V   R   A   W   V   G   F   S   576

1729  GTG CCC CTG GCG CAC CGC ATC ACG GCG GGC GCG GAC ATC CTG CTG ATG  1776
 577   V   P   L   A   H   R   I   T   A   G   A   D   I   L   L   M   592

1777  CCG TCG CGG TTC GAG CCG TGC GGG CTG AAC CAG CTC TAC GCC ATG GCG  1824
 593   P   S   R   F   E   P   C   G   L   N   Q   L   Y   A   M   A   608

1825  TAC GGG ACC GTG CCC GTG GTG CAC GCC GTG GGG GGG CTC CGG GAC ACG  1872
 609   Y   G   T   V   P   V   V   H   A   V   G   G   L   R   D   T   624

1873  GTG GCG CCG TTC GAC CCG TTC AAC GAC ACC GGG CTC GGG TGG ACG TTC  1920
 625   V   A   P   F   D   P   F   N   D   T   G   L   G   W   T   F   640

1921  GAC CGC GCG GAG GCG AAC CGG ATG ATC GAC GCG CTC TCG CAC TGC CTC  1968
 641   D   R   A   E   A   N   R   M   I   D   A   L   S   H   C   L   656

1969  ACC ACG TAC CGG AAC TAC AAG GAG AGC TGG CGC GCC TGC AGG GCG CGC  2016
 657   T   T   Y   R   N   Y   K   E   S   W   R   A   C   R   A   R   672

2017  GGC ATG GCC GAG GAC CTC AGC TGG GAC CAC GCC GCC GTG CTG TAT GAG  2064
 673   G   M   A   E   D   L   S   W   D   H   A   A   V   L   Y   E   688

2065  GAC GTG CTC GTC AAG GCG AAG TAC CAG TGG TGA GCG AAT TAA TTG GCG  2112
 689   D   V   L   V   K   A   K   Y   Q   W   *   A   N   *   L   A   704

2113  ACG CGA CGC CGC TCC TGT CGC AGG ACC TGG ACG TTA TTT AGA AGG CTC  2160
 705   T   R   R   R   S   C   R   R   T   W   T   L   F   R   R   L   720

2161  TTC TCC CTG GCG GCT TTG ATG CGT GCG TCG CAT TTG CGC CGG GCG GAC  2208
 721   F   S   L   A   A   L   M   R   A   S   H   L   R   R   A   D   736

2209  GGG CGA CGG TGG TTG GCC TAC CGC CTA CGT CGG CTG CGT GCC CTG GGA  2256
 737   G   R   R   W   L   A   Y   R   L   R   R   L   R   A   L   G   752

2257  ATT TGG GCG GGC ACG ATG ATG CCA CTG GGC ACC GGG CGC GGG GTA GTA  2304
 753   I   W   A   G   T   M   M   P   L   G   T   G   R   G   V   V   768

2305  TGA TAT GAA ACC GAC GGC GAT GGA GAT GAG GCG CAT GGC ATT TTC CCA  2352
 769   *   Y   E   T   D   G   D   G   D   E   A   H   G   I   F   P   784

2353  CTG ATA AAT GGG GAG TTG TAT GCT ACT TTA ATA TCG CCA CTC CTG TTA  2400
 785   L   I   N   G   E   L   Y   A   T   L   I   S   P   L   L   L   800

2401  GTA TTT ATA TTG ATG GCG GCC GC                                   2423
 801   V   F   I   L   M   A   A                                       807
```

Fig 50 continued

673	CTT	GGA	GAT	GTC	GTG	GGT	GCT	TTG	CCT	AAG	GCT	CTG	GCG	AGG	AGA	GGA	720
225	L	G	D	V	V	G	A	L	P	K	A	L	A	R	R	G	240
721	CAC	CGT	GTT	ATG	GTC	GTG	ATA	CCA	AGA	TAT	GGA	GAG	TAT	GCC	GAA	GCC	768
241	H	R	V	M	V	V	I	P	R	Y	G	E	Y	A	E	A	256
769	CGG	GAT	TTA	GGT	GTA	AGG	AGA	CGT	TAC	AAG	GTA	GCT	GGA	CAG	GAT	TCA	816
257	R	D	L	G	V	R	R	R	Y	K	V	A	G	Q	D	S	272
817	GAA	GTT	ACT	TAT	TTT	CAC	TCT	TAC	ATT	GAT	GGA	GTT	GAT	TTT	GTA	TTC	864
273	E	V	T	Y	F	H	S	Y	I	D	G	V	D	F	V	F	288
865	GTA	GAA	GCC	CCT	CCC	TTC	CGG	CAC	CGG	CAC	AAT	AAT	ATT	TAT	GGG	GGA	912
289	V	E	A	P	P	F	R	H	R	H	N	N	I	Y	G	G	304
913	GAA	AGA	TTG	GAT	ATT	TTG	AAG	CGC	ATG	ATT	TTG	TTC	TGC	AAG	GCC	GCT	960
305	E	R	L	D	I	L	K	R	M	I	L	F	C	K	A	A	320
961	GTT	GAG	GTT	CCA	TGG	TAT	GCT	CCA	TGT	GGC	GGT	ACT	GTC	TAT	GGT	GAT	1008
321	V	E	V	P	W	Y	A	P	C	G	G	T	V	Y	G	D	336
1009	GGC	AAC	TTA	GTT	TTC	ATT	GCT	AAT	GAT	TGG	CAT	ACC	GCA	CTT	CTG	CCT	1056
337	G	N	L	V	F	I	A	N	D	W	H	T	A	L	L	P	352
1057	GTC	TAT	CTA	AAG	GCC	TAT	TAC	CGG	GAC	AAT	GGT	TTG	ATG	CAG	TAT	GCT	1104
353	V	Y	L	K	A	Y	Y	R	D	N	G	L	M	Q	Y	A	368
1105	CGC	TCT	GTG	CTT	GTG	ATA	CAC	AAC	ATT	GCT	CAT	CAG	GGT	CGT	GGC	CCT	1152
369	R	S	V	L	V	I	H	N	I	A	H	Q	G	R	G	P	384
1153	GTA	GAC	GAC	TTC	GTC	AAT	TTT	GAC	TTG	CCT	GAA	CAC	TAC	ATC	GAC	CAC	1200
385	V	D	D	F	V	N	F	D	L	P	E	H	Y	I	D	H	400
1201	TTC	AAA	CTG	TAT	GAC	AAC	ATT	GGT	GGG	GAT	CAC	AGC	AAC	GTT	TTT	GCT	1248
401	F	K	L	Y	D	N	I	G	G	D	H	S	N	V	F	A	416
1249	GCG	GGG	CTG	AAG	ACG	GCA	GAC	CGG	GTG	GTG	ACC	GTT	AGC	AAT	GGC	TAC	1296
417	A	G	L	K	T	A	D	R	V	V	T	V	S	N	G	Y	432
1297	ATG	TGG	GAG	CTG	AAG	ACT	TCG	GAA	GGC	GGG	TGG	GGC	CTC	CAC	GAC	ATC	1344
433	M	W	E	L	K	T	S	E	G	G	W	G	L	H	D	I	448
1345	ATA	AAC	CAG	AAC	GAC	TGG	AAG	CTG	CAG	GGC	ATC	GTG	AAC	GGC	ATC	GAC	1392
449	I	N	Q	N	D	W	K	L	Q	G	I	V	N	G	I	D	464
1393	ATG	AGC	GAG	TGG	AAC	CCC	GCT	GTG	GAC	GTG	CAC	CTC	CAC	TCC	GAC	GAC	1440
465	M	S	E	W	N	P	A	V	D	V	H	L	H	S	D	D	480
1441	TAC	ACC	AAC	TAC	ACG	TTC	GAG	ACG	CTG	GAC	ACC	GGC	AAG	CGG	CAG	TGC	1488
481	Y	T	N	Y	T	F	E	T	L	D	T	G	K	R	Q	C	496
1489	AAG	GCC	GCC	CTG	CAG	CGG	CAG	CTG	GGC	CTG	CAG	GTC	CGC	GAC	GAC	GTG	1536
497	K	A	A	L	Q	R	Q	L	G	L	Q	V	R	D	D	V	512
1537	CCA	CTG	ATC	GGG	TTC	ATC	GGG	CGG	CTG	GAC	CAC	CAG	AAG	GGC	GTG	GAC	1584
513	P	L	I	G	F	I	G	R	L	D	H	Q	K	G	V	D	528
1585	ATC	ATC	GCC	GAC	GCG	ATC	CAC	TGG	ATC	GCG	GGG	CAG	GAC	GTG	CAG	CTG	1632
529	I	I	A	D	A	I	H	W	I	A	G	Q	D	V	Q	L	544

Fig 51

DNASIS ***** DNA TRANSLATION LIST ***** DATE 04-03-97

*** INPUT INFORMATION ***

FILE NAME : MSS2C.SEQ SEQUENCE : NORMAL 2007 BP

CODON TABLE : UNIV.TCN

SEQUENCE REGION : 1 - 2007

TRANSLATION REGION : 1 - 2007

*** DNA TRANSLATION ***

```
  1  GCT GAG GCT GAG GCC GGG GGC AAG GAC GCG CCG CCG GAG AGG AGC GGC   48
  1   A   E   A   E   A   G   G   K   D   A   P   P   E   R   S   G   16

 49  GAC GCC GCC AGG TTG CCC CGC GCT CGG CGC AAT GCG GTC TCC AAA CGG   96
 17   D   A   A   R   L   P   R   A   R   R   N   A   V   S   K   R   32

 97  AGG GAT CCT CTT CAG CCG GTC GGC CGG TAC GGC TCC GCG ACG GGA AAC  144
 33   R   D   P   L   Q   P   V   G   R   Y   G   S   A   T   G   N   48

145  ACG GCC AGG ACC GGC GCC GCG TCC TGC CAG AAC GCC GCA TTG GCG GAC  192
 49   T   A   R   T   G   A   A   S   C   Q   N   A   A   L   A   D   64

193  GTT GAG ATC GTT GAG ATC AAG TCC ATC GTC GCC GCG CCG CCG ACG AGC  240
 65   V   E   I   V   E   I   K   S   I   V   A   A   P   P   T   S   80

241  ATA GTG AAG TTC CCA GGG CGC GGG CTA CAG GAT GAT CCT TCC CTC TGG  288
 81   I   V   K   F   P   G   R   G   L   Q   D   D   P   S   L   W   96

289  GAC ATA GCA CCG GAG ACT GTC CTC CCA GCC CCG AAG CCA CTG CAT GAA  336
 97   D   I   A   P   E   T   V   L   P   A   P   K   P   L   H   E  112

337  TCG CCT GCG GTT GAC GGA GAT TCA AAT GGA ATT GCA CCT CCT ACA GTT  384
113   S   P   A   V   D   G   D   S   N   G   I   A   P   P   T   V  128

385  GAG CCA TTA GTA CAG GAG GCC ACT TGG GAT TTC AAG AAA TAC ATC GGT  432
129   E   P   L   V   Q   E   A   T   W   D   F   K   K   Y   I   G  144

433  TTT GAC GAG CCT GAC GAA GCG AAG GAT GAT TCC AGG GTT GGT GCA GAT  480
145   F   D   E   P   D   E   A   K   D   D   S   R   V   G   A   D  160

481  GAT GCT GGT TCT TTT GAA CAT TAT GGG ACA ATG ATT CTG GGC CTT TGT  528
161   D   A   G   S   F   E   H   Y   G   T   M   I   L   G   L   C  176

529  GGG GAG AAT GTT ATG AAC GTG ATC GTG GTG GCT GCT GAA TGT TCT CCA  576
177   G   E   N   V   M   N   V   I   V   V   A   A   E   C   S   P  192

577  TGG TGC AAA ACA GGT GGT CTT GGA GAT GTT GTG GGA GCT TTA CCC AAG  624
193   W   C   K   T   G   G   L   G   D   V   V   G   A   L   P   K  208

625  GCT TTA GCG AGA AGA GGA CAT CGT GTT ATG GTT GTG GTA CCA AGG TAT  672
209   A   L   A   R   R   G   H   R   V   M   V   V   V   P   R   Y  224
```

71/90 Fig 51 continued

673	GGG	GAC	TAT	GTG	GAA	GCC	TTT	GAT	ATG	GGA	ATC	CGG	AAA	TAC	TAC	AAA	720
225	G	D	Y	V	E	A	F	D	M	G	I	R	K	Y	Y	K	240
721	GCT	GCA	GGA	CAG	GAC	CTA	GAA	GTG	AAC	TAT	TTC	CAT	GCA	TTT	ATT	GAT	768
241	A	A	G	Q	D	L	E	V	N	Y	F	H	A	F	I	D	256
769	GGA	GTC	GAC	TTT	GTG	TTC	ATT	GAT	GCC	TCT	TTC	CGG	CAC	CGT	CAA	GAT	816
257	G	V	D	F	V	F	I	D	A	S	F	R	H	R	Q	D	272
817	GAC	ATA	TAT	GGG	GGA	AGT	AGG	CAG	GAA	ATC	ATG	AAG	CGC	ATG	ATT	TTG	864
273	D	I	Y	G	G	S	R	Q	E	I	M	K	R	M	I	L	288
865	TTT	TGC	AAG	GTT	GCT	GTT	GAG	GTT	CCT	TGG	CAC	GTT	CCA	TGC	GGT	GGT	912
289	F	C	K	V	A	V	E	V	P	W	H	V	P	C	G	G	304
913	GTG	TGC	TAC	GGA	GAT	GGA	AAT	TTG	GTG	TTC	ATT	GCC	ATG	AAT	TGG	CAC	960
305	V	C	Y	G	D	G	N	L	V	F	I	A	M	N	W	H	320
961	ACT	GCA	CTC	CTG	CCT	GTT	TAT	CTG	AAG	GCA	TAT	TAC	AGA	GAC	CAT	GGG	1008
321	T	A	L	L	P	V	Y	L	K	A	Y	Y	R	D	H	G	336
1009	TTA	ATG	CAG	TAC	ACT	CGC	TCC	GTC	CTC	GTC	ATA	CAT	AAC	ATC	GGC	CAC	1056
337	L	M	Q	Y	T	R	S	V	L	V	I	H	N	I	G	H	352
1057	CAG	GGC	CGT	GGT	CCT	GTA	CAT	GAA	TTC	CCG	TAC	ATG	GAC	TTG	CTG	AAC	1104
353	Q	G	R	G	P	V	H	E	F	P	Y	M	D	L	L	N	368
1105	ACT	AAC	CTT	CAA	CAT	TTC	GAG	CTG	TAC	GAT	CCC	GTC	GGT	GGC	GAG	CAC	1152
369	T	N	L	Q	H	F	E	L	Y	D	P	V	G	G	E	H	384
1153	GCC	AAC	ATC	TTT	GCC	GCG	TGT	GTT	CTG	AAG	ATG	GCA	GAC	CGG	GTG	GTG	1200
385	A	N	I	F	A	A	C	V	L	K	M	A	D	R	V	V	400
1201	ACT	GTC	AGC	CGC	GGC	TAC	CTG	TGG	GAG	CTG	AAG	ACA	GTG	GAA	GGC	GGC	1248
401	T	V	S	R	G	Y	L	W	E	L	K	T	V	E	G	G	416
1249	TGG	GGC	CTC	CAC	GAC	ATC	ATC	CGT	TCT	AAC	GAC	TGG	AAG	ATC	AAT	GGC	1296
417	W	G	L	H	D	I	I	R	S	N	D	W	K	I	N	G	432
1297	ATT	CGT	GAA	CGC	ATC	GAC	CAC	CAG	GAG	TGG	AAC	CCC	AAG	GTG	GAC	GTG	1344
433	I	R	E	R	I	D	H	Q	E	W	N	P	K	V	D	V	448
1345	CAC	CTG	CGG	TCG	GAC	GGC	TAC	ACC	AAC	TAC	TCC	CTC	GAG	ACA	CTC	GAC	1392
449	H	L	R	S	D	G	Y	T	N	Y	S	L	E	T	L	D	464
1393	GCT	GGA	AAG	CGG	CAG	TGC	AAG	GCG	GCC	CTG	CAG	CGG	GAC	GTG	GGC	CTG	1440
465	A	G	K	R	Q	C	K	A	A	L	Q	R	D	V	G	L	480
1441	GAA	GTG	CGC	GAC	GAC	GTG	CCG	CTG	CTC	GGC	TTC	ATC	GGG	CGT	CTG	GAT	1488
481	E	V	R	D	D	V	P	L	L	G	F	I	G	R	L	D	496
1489	GGA	CAG	AAG	GGC	GTG	GAC	ATC	ATC	GGG	GAC	GCG	ATG	CCG	TGG	ATC	GCG	1536
497	G	Q	K	G	V	D	I	I	G	D	A	M	P	W	I	A	512
1537	GGG	CAG	GAC	GTG	CAG	CTG	GTG	ATG	CTG	GGC	ACC	GGC	CCA	CCT	GAC	CTG	1584
513	G	Q	D	V	Q	L	V	M	L	G	T	G	P	P	D	L	528
1585	GAA	CGA	ATG	CTG	CAG	CAC	TTG	GAG	CGG	GAG	CAT	CCC	AAC	AAG	GTG	CGC	1632
529	E	R	M	L	Q	H	L	E	R	E	H	P	N	K	V	R	544

Fig 51 continued

1633	GGG	TGG	GTC	GGG	TTC	TCG	GTC	CTA	ATG	GTG	CAT	CGC	ATC	ACG	CCG	GGC	1680
545	G	W	V	G	F	S	V	L	M	V	H	R	I	T	P	G	560
1681	GCC	AGC	GTG	CTG	GTG	ATG	CCC	TCC	CGC	TTC	GCC	GGC	GGG	CTG	AAC	CAG	1728
561	A	S	V	L	V	M	P	S	R	F	A	G	G	L	N	Q	576
1729	CTC	TAC	GCG	ATG	GCA	TAC	GGC	ACC	GTC	CCT	GTG	GTG	CAC	GCC	GTG	GGC	1776
577	L	Y	A	M	A	Y	G	T	V	P	V	V	H	A	V	G	592
1777	GGG	CTC	AGG	GAC	ACC	GTG	GCG	CCG	TTC	GAC	CCG	TTC	GGC	GAC	GCC	GGG	1824
593	G	L	R	D	T	V	A	P	F	D	P	F	G	D	A	G	608
1825	CTC	GGG	TGG	ACT	TTT	GAC	CGC	GCC	GAG	GCC	AAC	AAG	CTG	ATC	GAG	GTG	1872
609	L	G	W	T	F	D	R	A	E	A	N	K	L	I	E	V	624
1873	CTC	AGC	CAC	TGC	CTC	GAC	ACG	TAC	CGA	AAC	TAC	GAG	GAG	AGC	TGG	AAG	1920
625	L	S	H	C	L	D	T	Y	R	N	Y	E	E	S	W	K	640
1921	AGT	CTC	CAG	GCG	CGC	GGC	ATG	TCG	CAG	AAC	CTC	AGC	TGG	GAC	CAC	GCG	1968
641	S	L	Q	A	R	G	M	S	Q	N	L	S	W	D	H	A	656
1969	GCT	GAG	CTC	TAC	GAG	GAC	GTC	CTT	GTC	AAG	TAC	CAG	TGG				2007
657	A	E	L	Y	E	D	V	L	V	K	Y	Q	W				669

Fig 52

DNASIS ***** DNA TRANSLATION LIST ***** DATE 04-03-97

*** INPUT INFORMATION ***

FILE NAME : MSS1B.SEQ SEQUENCE : NORMAL 1749 BP

CODON TABLE : UNIV.TCN

SEQUENCE REGION : 1 - 1749

TRANSLATION REGION : 1 - 1749

*** DNA TRANSLATION ***

1	TGC	GTC	GCG	GAG	CTG	AGC	AGG	GAG	GGG	CCC	GCG	CCG	CGC	CCG	CTG	CCA	48
1	C	V	A	E	L	S	R	E	G	P	A	P	R	P	L	P	16
49	CCC	GCG	CTG	CTG	GCG	CCC	CCG	CTC	GTG	CCC	GGC	TTC	CTC	GCG	CCG	CCG	96
17	P	A	L	L	A	P	P	L	V	P	G	F	L	A	P	P	32
97	GCC	GAG	CCC	ACG	GGT	GAG	CCG	GCA	TCG	ACG	CCG	CCG	CCC	GTG	CCC	GAC	144
33	A	E	P	T	G	E	P	A	S	T	P	P	P	V	P	D	48
145	GCC	GGC	CTG	GGG	GAC	CTC	GGT	CTC	GAA	CCT	GAA	GGG	ATT	GCT	GAA	GGT	192
49	A	G	L	G	D	L	G	L	E	P	E	G	I	A	E	G	64
193	TCC	ATC	GAT	AAC	ACA	GTA	GTT	GTG	GCA	AGT	GAG	CAA	GAT	TCT	GAG	ATT	240
65	S	I	D	N	T	V	V	V	A	S	E	Q	D	S	E	I	80
241	GTG	GTT	GGA	AAG	GAG	CAA	GCT	CGA	GCT	AAA	GTA	ACA	CAA	AGC	ATT	GTC	288
81	V	V	G	K	E	Q	A	R	A	K	V	T	Q	S	I	V	96
289	TTT	GTA	ACC	GGC	GAA	GCT	TCT	CCT	TAT	GCA	AAG	TCT	GGG	GGT	CTA	GGA	336
97	F	V	T	G	E	A	S	P	Y	A	K	S	G	G	L	G	112
337	GAT	GTT	TGT	GGT	TCA	TTG	CCA	GTT	GCT	CTT	GCT	GCT	CGT	GGT	CAC	CGT	384
113	D	V	C	G	S	L	P	V	A	L	A	A	R	G	H	R	128
385	GTG	ATG	GTT	GTA	ATG	CCC	AGA	TAT	TTA	AAT	GGT	ACC	TCC	GAT	AAG	AAT	432
129	V	M	V	V	M	P	R	Y	L	N	G	T	S	D	K	N	144
433	TAT	GCA	AAT	GCA	TTT	TAC	ACA	GAA	AAA	CAC	ATT	CGG	ATT	CCA	TGC	TTT	480
145	Y	A	N	A	F	Y	T	E	K	H	I	R	I	P	C	F	160
481	GGC	GGT	GAA	CAT	GAA	GTT	ACC	TTC	TTC	CAT	GAG	TAT	AGA	GAT	TCA	GTT	528
161	G	G	E	H	E	V	T	F	F	H	E	Y	R	D	S	V	176
529	GAC	TGG	GTG	TTT	GTT	GAT	CAT	CCC	TCA	TAT	CAC	AGA	CCT	GGA	AAT	TTA	576
177	D	W	V	F	V	D	H	P	S	Y	H	R	P	G	N	L	192
577	TAT	GGA	GAT	AAG	TTT	GGT	GCT	TTT	GGT	GAT	AAT	CAG	TTC	AGA	TAC	ACA	624
193	Y	G	D	K	F	G	A	F	G	D	N	Q	F	R	Y	T	208
625	CTC	CTT	TGC	TAT	GCT	GCA	TGT	GAG	GCT	CCT	TTG	ATC	CTT	GAA	TTG	GGA	672
209	L	L	C	Y	A	A	C	E	A	P	L	I	L	E	L	G	224

Fig 52 continued

673	GGA	TAT	ATT	TAT	GGA	CAG	AAT	TGC	ATG	TTT	GTT	GTC	AAT	GAT	TGG	CAT	720
225	G	Y	I	Y	G	Q	N	C	M	F	V	V	N	D	W	H	240
721	GCC	AGT	CTA	GTG	CCA	GTC	CTT	CTT	GCT	GCA	AAA	TAT	AGA	CCA	TAT	GGT	768
241	A	S	L	V	P	V	L	L	A	A	K	Y	R	P	Y	G	256
769	GTT	TAT	AAA	GAC	TCC	CGC	AGC	ATT	CTT	GTA	ATA	CAT	AAT	TTA	GCA	CAT	816
257	V	Y	K	D	S	R	S	I	L	V	I	H	N	L	A	H	272
817	CAG	GGT	GTA	GAG	CCT	GCA	AGC	ACA	TAT	CCT	GAC	CTT	GGG	TTG	CCA	CCT	864
273	Q	G	V	E	P	A	S	T	Y	P	D	L	G	L	P	P	288
865	GAA	TGG	TAT	GGA	GCT	CTG	GAG	TGG	GTA	TTC	CCT	GAA	TGG	GCG	AGG	AGG	912
289	E	W	Y	G	A	L	E	W	V	F	P	E	W	A	R	R	304
913	CAT	GCC	CTT	GAC	AAG	GGT	GAG	GCA	GTT	AAT	TTT	TTG	AAA	GGT	GCA	GTT	960
305	H	A	L	D	K	G	E	A	V	N	F	L	K	G	A	V	320
961	GTG	ACA	GCA	GAT	CGA	ATC	GTG	ACT	GTC	AGT	AAG	GGT	TAT	TCG	TGG	GAG	1008
321	V	T	A	D	R	I	V	T	V	S	K	G	Y	S	W	E	336
1009	GTC	ACA	ACT	GCT	GAA	GGT	GGA	CAG	GGC	CTC	AAT	GAG	CTC	TTA	AGC	TCC	1056
337	V	T	T	A	E	G	G	Q	G	L	N	E	L	L	S	S	352
1057	AGA	AAG	AGT	GTA	TTA	AAC	GGA	ATT	GTA	AAT	GGA	ATT	GAC	ATT	AAT	GAT	1104
353	R	K	S	V	L	N	G	I	V	N	G	I	D	I	N	D	368
1105	TGG	AAC	CCT	GCC	ACA	GAC	AAA	TGT	ATC	CCC	TGT	CAT	TAT	TCT	GTT	GAT	1152
369	W	N	P	A	T	D	K	C	I	P	C	H	Y	S	V	D	384
1153	GAC	CTC	TCT	GGA	AAG	GCC	AAA	TGT	AAA	GGT	GCA	TTG	CAG	AAG	GAG	CTG	1200
385	D	L	S	G	K	A	K	C	K	G	A	L	Q	K	E	L	400
1201	GGT	TTA	CCT	ATA	AGG	CCT	GAT	GTT	CCT	CTG	ATT	GGC	TTT	ATT	GGA	AGG	1248
401	G	L	P	I	R	P	D	V	P	L	I	G	F	I	G	R	416
1249	TTG	GAT	TAT	CAG	AAA	GGC	ATT	GAT	CTC	ATT	CAA	CTT	ATC	ATA	CCA	GAT	1296
417	L	D	Y	Q	K	G	I	D	L	I	Q	L	I	I	P	D	432
1297	CTC	ATG	CGG	GAA	GAT	GTT	CAA	TTT	GTC	ATG	CTT	GGA	TCT	GGT	GAC	CCA	1344
433	L	M	R	E	D	V	Q	F	V	M	L	G	S	G	D	P	448
1345	GAG	CTT	GAA	GAT	TGG	ATG	AGA	TCT	ACA	GAG	TCG	ATC	TTC	AAG	GAT	AAA	1392
449	E	L	E	D	W	M	R	S	T	E	S	I	F	K	D	K	464
1393	TTT	CGT	GGA	TGG	GTT	GGA	TTT	AGT	GTT	CCA	GTT	TCC	CAC	CGA	ATA	ACT	1440
465	F	R	G	W	V	G	F	S	V	P	V	S	H	R	I	T	480
1441	GCC	GGC	TGC	GAT	ATA	TTG	TTA	ATG	CCA	TCC	AGA	TTC	GAA	CCT	TGT	GGT	1488
481	A	G	C	D	I	L	L	M	P	S	R	F	E	P	C	G	496
1489	CTC	AAT	CAG	CTA	TAT	GCT	ATG	CAG	TAT	GGC	ACA	GTT	CCT	GTT	GTC	CAT	1536
497	L	N	Q	L	Y	A	M	Q	Y	G	T	V	P	V	V	H	512
1537	GCA	ACT	GGG	GGC	CTT	AGA	GAT	ACC	GTG	GAG	AAC	TTC	AAC	CCT	TTC	GGT	1584
513	A	T	G	G	L	R	D	T	V	E	N	F	N	P	F	G	528
1585	GAG	AAT	GGA	GAG	CAG	GGT	ACA	GGG	TGG	GCA	TTC	GCA	CCC	CTA	ACC	ACA	1632
529	E	N	G	E	Q	G	T	G	W	A	F	A	P	L	T	T	544

Fig 52 continued

1633	GAA	AAC	ATG	TTT	GTG	GAC	ATT	GCG	AAC	TGC	AAT	ATC	TAC	ATA	CAG	GGA	1680
545	E	N	M	F	V	D	I	A	N	C	N	I	Y	I	Q	G	560
1681	ACA	CAA	GTC	CTC	CTG	GGA	AGG	GCT	AAT	GAA	GCG	AGG	CAT	GTC	AAA	AGA	1728
561	T	Q	V	L	L	G	R	A	N	E	A	R	H	V	K	R	576
1729	CTT	CAC	GTG	GGA	CCA	TGC	CGC										1749
577	L	H	V	G	P	C	R										583

Fig 53

TRANSLATE of: mb73be2.seq check: 2027 from: 6 to: 2640
generated symbols 1 to: 878.

LOCUS MZEGLUCTRN 2640 bp ss-mRNA PLN May 20,1993
DEFINITION Maize starch branching enzyme II mRNA, complete cds.

KEYWORDS 1,4-alpha-glucan branching enzyme; amylo-transglycosylase; glucanotransferase; starch branching enzyme II.
SOURCE Zea mays (cultivar B73) 30 days post pollenation endosperm . . .

Mb73be2.pep Length: 799 Transit peptide 1-61 May 20, 1993 13:29 Type: P
Check: 2844 ..

```
  1 MAFRVSGAVL GGAVRAPRLT GGGEGSLVFR HTGLFLTRGA RVGCSGTHGA
 51 MRAAAAARKA VMVPEGENDG LASRADSAQF QSDELEVPDI SEETTCGAGV
101 ADAQALNRVR VVPPPSDGQK IFQIDPMLQG YKYHLEYRYS LYRRIRSDID
151 EHEGGLEAFS RSYEKFGFNR SAEGITYREW APGAFSAALV GDFNNWDPNA
201 DRMSKNEFGV WEIFLPNNAD GTSPIPHGSR VKVRMDTPSG IKDSIPAWIK
251 YSVQAPGEIP YDGIYYDPPE EVKYVFRHAQ PKRPKSLRIY ETHVGMSSPE
301 PKINTYVNFR DEVLPRIKKL GYNAVQIMAI QEHSYYGSFG YHVTNFFAPS
351 SRFGTPEELK SLIDRAHELG LLVLMDVVHS HASSNTLDGL NGFDGTDTHY
401 FHSGPRGHHW MWDSRLFNYG NWEVLRFLLS NARWWLEEYK FDGFRFDGVT
451 SMMYTHHGLQ VTFTGNFNEY FGFATDVDAV VYLMLVNDLI HGLYPEAVTI
501 GEDVSGMPTF ALPVHDGGVG FDYRMHMAVA DKWIDLLKQS DETWKMGDIV
551 HTLTNRRWLE KCVTYAESHD QALVGDKTIA FWLMDKDMYD FMALDRPSTP
601 TIDRGIALHK MIRLITMGLG GEGYLNFMGN EFGHPEWIDF PRGPQRLPSG
651 KFIPGNNNSY DKCRRRFDLG DADYLRYHGM QEFDQAMQHL EQKYEFMTSD
701 HQYISRKHEE DKVIVFEKGD LVFVFNFHCN NSYFDYRIGC RKPGVYKVVL
751 DSDAGLFGGF SRIHHAAEHF TADCSHDNRP YSFSVYTPSR TCVVYAPVE*
801 *RGTRCCAAC VGLSM*GKTF FQNRQMHACM LQ*GSDTLID AGKPMHLAAL
851 SSLFI*DLQG VN*T*SFRFS LKKKKKKL
```

Fig 53 continued

```
LOCUS       MZEGLUCTRN    2640 bp ss-mRNA            PLN       May 20,1993
DEFINITION  Maize starch branching enzyme II mRNA, complete cds.

KEYWORDS    1,4-alpha-glucan branching enzyme; amylo-transglycosylase;
            glucanotransferase; starch branching enzyme II.
SOURCE      Zea mays (cultivar B73) 30 days post pollenation endosperm
            cDNA to mRNA.
  ORGANISM  Zea mays

  STANDARD  full automatic
FEATURES             Location/Qualifiers
     sig_peptide     6. .188
                     /codon_start=3
     mat_peptide     189. .2402
                     /product="starch branching enzyme II"
                     /codon_start=3
     CDS             6. .2402
                     /EC_number="2.4.1.18"
                     /product="starch branching enzyme II"
                     /codon_start=3
mb73be2.seq  Length: 2640  May 20, 1993  13:28  Type: N  Check: 2027
..
GCGAGATGGCGTTCCGGGTTTCTGGGGCGGTGCTCGGTGGGGCCGTAAGGGCTCCCCGACTCACCGGCGGCGGGG
AGGGTAGTCTAGTCTTCCGGCACACCGGCCTCTTCTTAACTCGGGGTGCTCGAGTTGGATGTTCGGGGACGCACG
GGGCCATGCGCGCGGCGGCCGCGGCCAGGAAGGCGGTCATGGTTCCTGAGGGCGAGAATGATGGCCTCGCATCAA
GGGCTGACTCGGCTCAATTCCAGTCGGATGAACTGGAGGTACCAGACATTTCTGAAGAGACAACGTGCGGTGCTG
GTGTGGCTGATGCTCAAGCCTTGAACAGAGTTCGAGTGGTCCCCCCACCAAGCGATGGACAAAAAATATTCCAGA
TTGACCCCATGTTGCAAGGCTATAAGTACCATCTTGAGTATCGGTACAGCCTCTATAGAAGAATCCGTTCAGACA
TTGATGAACATGAAGGAGGCTTGGAAGCCTTCTCCCGTAGTTATGAGAAGTTTGGATTTAATCGCAGCGCGGAAG
GTATCACATATCGAGAATGGGCTCCTGGAGCATTTTCTGCAGCATTGGTGGGTGACTTCAACAACTGGGATCCAA
ATGCAGATCGTATGAGCAAAAATGAGTTTGGTGTTTGGAAATTTTTCTGCCTAACAATGCAGATGGTACATCAC
CTATTCCTCATGGATCTCGTGTAAAGGTGAGAATGGATACTCCATCAGGGATAAAGGATTCAATTCCAGCCTGGA
TCAAGTACTCAGTGCAGGCCCCAGGAGAAATACCATATGATGGGATTTATTATGATCCTCCTGAAGAGGTAAAGT
ATGTGTTCAGGCATGCGCAACCTAAACGACCAAAATCATTGCGGATATATGAAACACATGTCGGAATGAGTAGCC
CGGAACCGAAGATAAACACATATGTAAACTTTAGGGATGAAGTCCTCCCAAGAATAAAAAAACTTGGATACAATG
CAGTGCAAATAATGGCAATCCAAGAGCACTCATATTATGGAAGCTTTGGATACCATGTAACTAATTTTTTTGCGC
CAAGTAGTCGTTTTGGTACCCCAGAAGAATTGAAGTCTTTGATTGATAGAGCACATGAGCTTGGTTTGCTAGTTC
TCATGGATGTGGTTCATAGTCATGCGTCAAGTAATACTCTGGATGGGTTGAATGGTTTTGATGGTACAGATACAC
ATTACTTTCACAGTGGTCCACGTGGCCATCACTGGATGTGGGATTCTCGCCTATTTAACTATGGGAACTGGGAAG
TTTTAAGATTTCTTCTCTCCAATGCTAGATGGTGGCTCGAGGAATATAAGTTTGATGGTTTCCGTTTTGATGGTG
TGACCTCCATGATGTACACTCATCACGGATTACAAGTAACATTTACGGGGAACTTCAATGAGTATTTTGGCTTTG
CCACCGATGTAGATGCAGTGGTTTACTTGATGCTGGTAAATGATCTAATTCATGGACTTTATCCTGAGGCTGTAA
CCATTGGTGAAGATGTTAGTGGAATGCCTACATTTGCCCTTCCTGTTCACGATGGTGGGGTAGGTTTTGACTATC
GGATGCATATGGCTGTGGCTGACAAATGGATTGACCTTCTCAAGCAAAGTGATGAAACTTGGAAGATGGGTGATA
TTGTGCACACACTGACAAATAGGAGGTGGTTAGAGAAGTGTGTAACTTATGCTGAAAGTCATGATCAAGCATTAG
TCGGCGACAAGACTATTGCGTTTTGGTTGATGGACAAGGATATGTATGATTTCATGGCCCTCGATAGACCTTCAA
CTCCTACCATTGATCGTGGGATAGCATTACATAAGATGATTAGACTTATCACAATGGGTTTAGGAGGAGAGGGCT
ATCTTAATTTCATGGGAAATGAGTTTGGACATCCTGAATGGATAGATTTTCCAAGAGGTCCGCAAAGACTTCCAA
GTGGTAAGTTTATTCCAGGGAATAACAACAGTTATGACAAATGTCGTCGAAGATTTGACCTGGGTGATGCAGACT
ATCTTAGGTATCATGGTATGCAAGAGTTTGATCAGGCAATGCAACATCTTGAGCAAAAATATGAATTCATGACAT
CTGATCACCAGTATATTTCCCGGAAACATGAGGAGGATAAGGTGATTGTGTTCGAAAAGGGAGATTTGGTATTTG
TGTTCAACTTCCACTGCAACAACAGCTATTTTGACTACCGTATTGGTTGTCGAAAGCCTGGGGTGTATAAGGTGG
TCTTGGACTCCGACGCTGGACTATTTGGTGGATTTAGCAGGATCCATCACGCAGCCGAGCACTTCACCGCCGACT
GTTCGCATGATAATAGGCCATATTCATTCTCGGTTTATACACCAAGCAGAACATGTGTCGTCTATGCTCCAGTGG
AGTGATAGCGGGGTACTCGTTGCTGCGCGGCATGTGTGGGGCTGTCGATGTGAGGAAAAACCTTCTTCCAAAACC
GGCAGATGCATGCATGCATGCTACAATAAGGTTCTGATACTTTAATCGATGCTGGAAAGCCCATGCATCTCGCTG
CGTTGTCCTCTCTATTTATATAAGACCTTCAAGGTGTCAATTAAACATAGAGTTTTCGTTTTTCGCTTAAAAAAA
AAAAAAAAAAACTCA
```

Fig 54

Maize Branching Enzyme I Mature Protein Sequence

1	ATVQEDKTMA	TAKGDVDHLP	IYDLDPKLEI	FKDHFRYRMK	RFLEQKGSIE	51
	ENEGSLESFS	KGYLKFGINT	NEDGTVYREW	APAAQEAELI	GDFNDWNGAN	101
	HKMEKDKFGV	WSIKIDHVKG	KPAIPHNSKV	KFRFLHGGVW	VDRIPALIRY	151
	ATVDASKFGA	PYDGVHWDPP	ASERYTFKHP	RPSKPAAPRI	YEAHVGMSGE	201
	KPAVSTYREF	ADNVLPRIRA	NNYNTVQLMA	VMEHSYYASF	GYHVTNFFAV	251
	SSRSGTPEDL	KYLVDKAHSL	GLRVLMDVVH	SHASNNVTDG	LNGYDVGQST	301
	QESYFHAGDR	GYHKLWDSRL	FNYANWEVLR	FLLSNLRYWL	DEFMFDGFRF	351
	DGVTSMLYHH	HGINVGFTGN	YQEYFSLDTA	VDAVVYMMLA	NHLMHKLLPE	401
	ATVVAEDVSG	MPVLCRPVDE	GGVGFDYRLA	MAIPDRWIDY	LKNKDDSEWS	451
	MGEIAHTLTN	RRYTEKCIAY	AESHDQSIVG	DKTIAFLLMD	KEMYTGMSDL	501
	QPASPTIDRG	IALQKMIHFI	TMALGGDGYL	NFMGNEFGHP	EWIDFPREGN	551
	NWSYDKCRRQ	WSLVDTDHLR	YKYMNAFDQA	MNALDERFSF	LSSSKQIVSD	601
	MNDEEKVIVF	ERGDLVFVFN	FHPKKTYEGY	KVGCDLPGKY	RVALDSDALV	651
	FGGHGRVGHD	VDHFTSPEGV	PGVPETNFNN	RPNSFKVLSP	PRTCVAYYRV	701
	DEAGAGRRLH	AKAETGKTSP	AESIDVKASR	ASSKEDKEAT	AGGKKGWKFA	751
	RQPSDQDTK					

79/90 Fig 54 continued

```
ID   D11081     unannotated; RNA; UNA; 2763 BP.
XX
AC   D11081;
XX
DT   13-JUL-1992 (Rel. 32, Created)
DT   13-JUL-1992 (Rel. 32, Last updated, Version 1)
XX
DE   Zea mays L. maize branching enzyme-I cDNA to mRNA.
XX
FH   Key             Location/Qualifiers
FH
XX
SQ   Sequence 2763 BP; 719 A; 585 C; 737 G; 722 T; 0 other;
D11081  Length: 2763  February 4, 1993  11:16  Type: N  Check: 6169  ..
       1  GCTGTGCCTC GTGTCGCCCT CTTCCTCGCC GACTCCGCTT CCGCCGCCGC
      51  GGCGCTCTCG CTCGCATGCT GATCGGGCGG CACCGCCGGG GATCGCGGGT
     101  GGCGGCAATG TGCGCCTGAG TGTGTTGTCT GTCCAGTGCA AGGCTCGCCG
     151  GTCAGGGGTG CGGAAGGTCA AGAGCAAATT CGCCACTGCA GCTACTGTGC
     201  AAGAAGATAA AACTATGGCA ACTGCCAAAG GCGATGTCGA CCATCTCCCC
     251  ATATACGACC TGGACCCCAA GCTGGAGATA TTCAAGGACC ATTTCAGGTA
     301  CCGGATGAAA AGATTCCTAG AGCAGAAAGG ATCAATTGAA GAAAATGAGG
     351  GAAGTCTTGA ATCTTTTTCT AAAGGCTATT TGAAATTTGG GATTAATACA
     401  AATGAGGATG GAACTGTATA TCGTGAATGG GCACCTGCTG CGCAGGAGGC
     451  AGAGCTTATT GGTGACTTCA ATGACTGGAA TGGTGCAAAC CATAAGATGG
     501  AGAAGGATAA ATTTGGTGTT TGGTCGATCA AAATTGACCA TGTCAAAGGG
     551  AAACCTGCCA TCCCTCACAA TTCCAAGGTT AAATTTCGCT TTCTACATGG
     601  TGGAGTATGG GTTGATCGTA TTCCAGCATT GATTCGTTAT GCGACTGTTG
     651  ATGCCTCTAA ATTTGGAGCT CCCTATGATG GTGTTCATTG GGATCCTCCT
     701  GCTTCTGAAA GGTACACATT TAAGCATCCT CGGCCTTCAA AGCCTGCTGC
     751  TCCACGTATC TATGAAGCCC ATGTAGGTAT GAGTGGTGAA AAGCCAGCAG
     801  TAAGCACATA TAGGGAATTT GCAGACAATG TGTTGCCACG CATACGAGCA
     851  AATAACTACA ACACAGTTCA GTTGATGGCA GTTATGGAGC ATTCGTACTA
     901  TGCTTCTTTC GGGTACCATG TGACAAATTT CTTTGCGGTT AGCAGCAGAT
     951  CAGGCACACC AGAGGACCTC AAATATCTTG TTGATAAGGC ACACAGTTTG
    1001  GGTTTGCGAG TTCTGATGGA TGTTGTCCAT AGCCATGCAA GTAATAATGT
```

Fig 54 continued

```
1101  ATTTTCATGC GGGAGATAGA GGTTATCATA AACTTTGGGA TAGTCGGCTG
1151  TTCAACTATG CTAACTGGGA GGTATTAAGG TTTCTTCTTT CTAACCTGAG
1201  ATATTGGTTG GATGAATTCA TGTTTGATGG CTTCCGATTT GATGGAGTTA
1251  CATCAATGCT GTATCATCAC CATGGTATCA ATGTGGGGTT TACTGGAAAC
1301  TACCAGGAAT ATTTCAGTTT GGACACAGCT GTGGATGCAG TTGTTTACAT
1351  GATGCTTGCA AACCATTTAA TGCACAAACT CTTGCCAGAA GCAACTGTTG
1401  TTGCTGAAGA TGTTTCAGGC ATGCCGGTCC TTTGCCGGCC AGTTGATGAA
1451  GGTGGGGTTG GGTTTGACTA TCGCCTGGCA ATGGCTATCC CTGATAGATG
1501  GATTGACTAC CTGAAGAATA AAGATGACTC TGAGTGGTCG ATGGGTGAAA
1551  TAGCGCATAC TTTGACTAAC AGGAGATATA CTGAAAAATG CATCGCATAT
1601  GCTGAGAGCC ATGATCAGTC TATTGTTGGC GACAAAACTA TTGCATTTCT
1651  CCTGATGGAC AAGGAAATGT ACACTGGCAT GTCAGACTTG CAGCCTGCTT
1701  CACCTACAAT TGATCGAGGG ATTGCACTCC AAAAGATGAT TCACTTCATC
1751  ACAATGGCCC TTGGAGGTGA TGGCTACTTG AATTTTATGG GAAATGAGTT
1801  TGGTCACCCA GAATGGATTG ACTTTCCAAG AGAAGGGAAC AACTGGAGCT
1851  ATGATAAATG CAGACGACAG TGGAGCCTTG TGGACACTGA TCACTTGCGG
1901  TACAAGTACA TGAATGCGTT TGACCAAGCG ATGAATGCGC TCGATGAGAG
1951  ATTTTCCTTC CTTTCGTCGT CAAAGCAGAT CGTCAGCGAC ATGAACGATG
2001  AGGAAAAGGT TATTGTCTTT GAACGTGGAG ATTTAGTTTT TGTTTTCAAT
2051  TTCCATCCCA AGAAAACTTA CGAGGGCTAC AAAGTGGGAT GCGATTTGCC
2101  TGGGAAATAC AGAGTAGCCC TGGACTCTGA TGCTCTGGTC TTCGGTGGAC
2151  ATGGAAGAGT TGGCCACGAC GTGGATCACT TCACGTCGCC TGAAGGGGTG
2201  CCAGGGGTGC CCGAAACGAA CTTCAACAAC CGGCCGAACT CGTTCAAAGT
2251  CCTTTCTCCG CCCCGCACCT GTGTGGCTTA TTACCGTGTA GACGAAGCAG
2301  GGGCTGGACG ACGTCTTCAC GCGAAAGCAG AGACAGGAAA GACGTCTCCA
2351  GCAGAGAGCA TCGACGTCAA AGCTTCCAGA GCTAGTAGCA AAGAAGACAA
2401  GGAGGCAACG GCTGGTGGCA AGAAGGGATG GAAGTTTGCG CGGCAGCCAT
2451  CCGATCAAGA TACCAAATGA AGCCACGAGT CCTTGGTGAG GACTGGACTG
2501  GCTGCCGGCG CCCTGTTAGT AGTCCTGCTC TACTGGACTA GCCGCCGCTG
2551  GCGCCCTTGG AACGGTCCTT TCCTGTAGCT TGCAGGCGAC TGGTGTCTCA
```

Fig 54 continued

2601 TCACCGAGCA GGCAGGCACT GCTTGTATAG CTTTTCTAGA ATAATAATCA
2651 GGGATGGATG GATGGTGTGT ATTGGCTATC TGGCTAGACG TGCATGTGCC
2701 CAGTTTGTAT GTACAGGAGC AGTTCCCGTC CAGAATAAAA AAAAACTTGT
2751 TGGGGGGTTT TTC

Fig 55

DNASIS ***** DNA TRANSLATION LIST ***** DATE 04-03-97

*** INPUT INFORMATION ***

FILE NAME : MSTS1TP.SEQ SEQUENCE : NORMAL 153 BP

CODON TABLE : UNIV.TCN

SEQUENCE REGION : 1 - 153

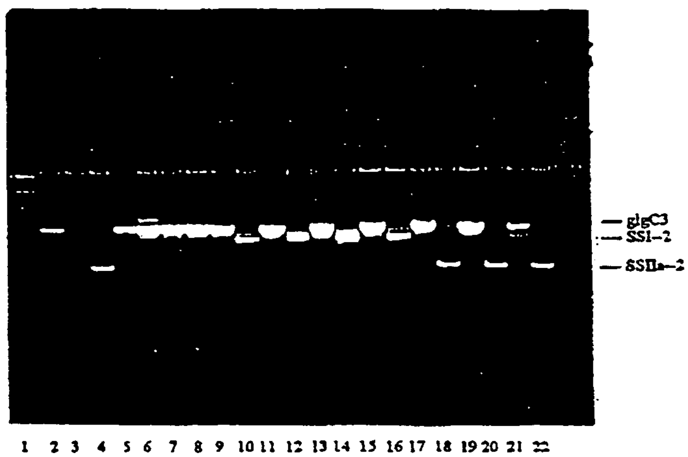
TRANSLATION REGION : 1 - 153

*** DNA TRANSLATION ***

1	ATG	GCG	ACG	CCC	TCG	GCC	GTG	GGC	GCC	GCG	TGC	CTC	CTC	CTC	GCG	CGG	48
1	M	A	T	P	S	A	V	G	A	A	C	L	L	L	A	R	16
49	GCC	GCC	TGG	CCG	GCC	GCC	GTC	GGC	GAC	CGG	GCG	CGC	CCG	CGG	CGG	CTC	96
17	A	A	W	P	A	A	V	G	D	R	A	R	P	R	R	L	32
97	CAG	CGC	GTG	CTG	CGC	CGC	CGG	TGC	GTC	GCG	GAG	CTG	AGC	AGG	GAG	GGG	144
33	Q	R	V	L	R	R	R	C	V	A	E	L	S	R	E	G	48
145	CCC	CAT	ATG														153
49	P	H	M														51

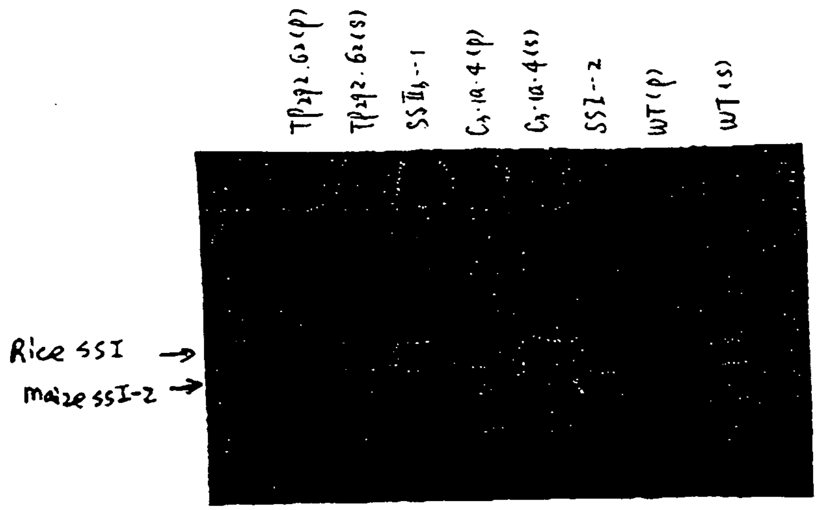
Transit peptide

Fig. 56



1. Marker,
2. PCR of WT DNA for glgC3;
3. PCR of WT DNA for SSI-2;
4. PCR of WT DNA for SSIIa-2;
5. PCR of C3-6 DNA for glgC3;
6. PCR of C3-9 DNA for glgC3;
7. PCR of C3-29 DNA for glgC3;
8. PCR of C3-35 DNA for glgC3;
9. PCR of C3-Ia-2 DNA for glgC3;
10. PCR of C3-Ia-2 DNA for SSI-2;
11. PCR of C3-Ia-3 DNA for glgC3;
12. PCR of C3-Ia-3 DNA for SSI-2;
13. PCR of C3-Ia-4 DNA for glgC3;
14. PCR of C3-Ia-4 DNA for SSI-2;
15. PCR of C3-Ia-5 DNA for glgC3;
16. PCR of C3-Ia-5 DNA for SSI-2;
17. PCR of C3-IIa-2 DNA for glgC3;
18. PCR of C3-IIa-2 DNA for SSIIa-2;
19. PCR of C3-IIa-5 DNA for glgC3;
20. PCR of C3-IIa-5 DNA for SSIIa-2;
21. PCR of C3-IIa-12 DNA for glgC3;
22. PCR of C3-IIa-12 DNA for SSIIa-2.

Fig 57


TP292.62(P)
TP292.62(S)
SSIIb-1
C5.1a.4(P)
C5.1a.4(S)
SSI-2
WT(P)
WT(S)
Rice SSI
maize SSI-2

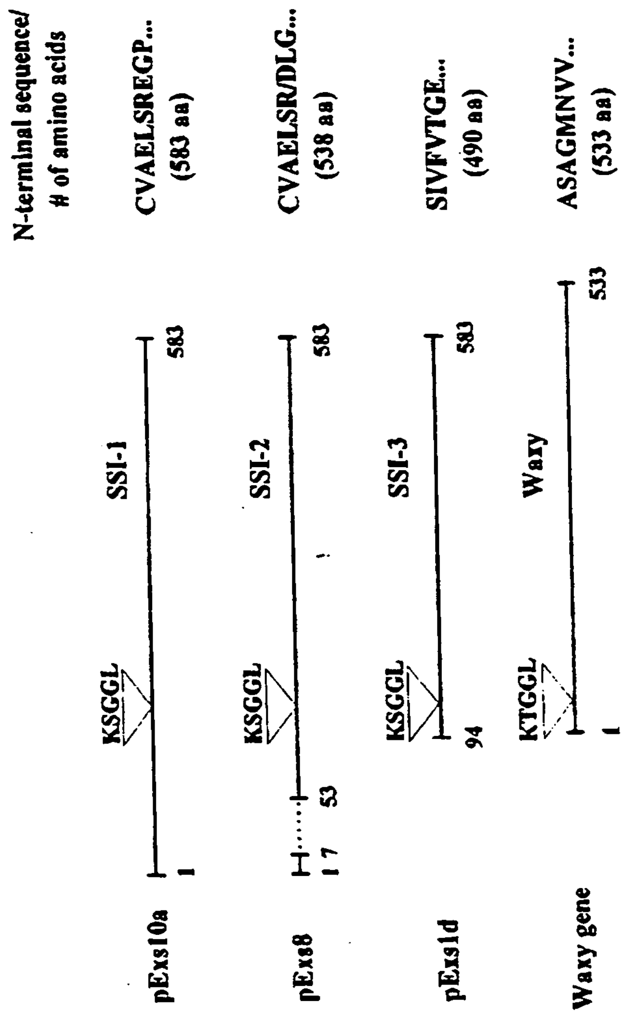
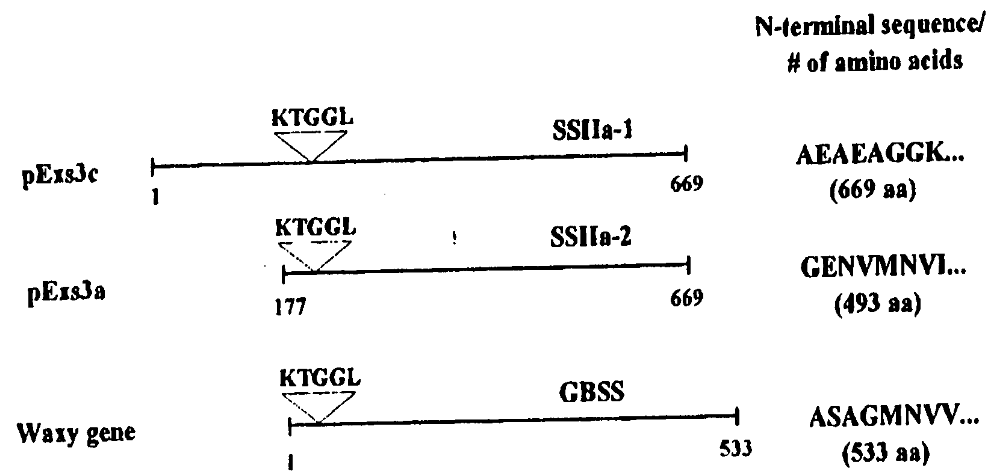

N-terminal sequence/
# of amino acids
pEx10a
1
KSGGL
SSI-1
583
CVAELSREGP...
(583 aa)
pEx8
1 7
53
KSGGL
SSI-2
583
CVAELSR/DLG...
(538 aa)
pExs1d
94
KSGGL
SSI-3
583
SIVFVTGE...
(490 aa)
Waxy gene
1
KTGGL
Waxy
533
ASAGMNVV...
(533 aa)

Fig. 58

Fig. 59


N-terminal sequence/
# of amino acids
pExs3c
KTGGL
SSIIa-1
1
669
AEAEAGGK...
(669 aa)
pExs3a
KTGGL
SSIIa-2
177
669
GENVMNVI...
(493 aa)
Waxy gene
KTGGL
GBSS
1
533
ASAGMNVV...
(533 aa)

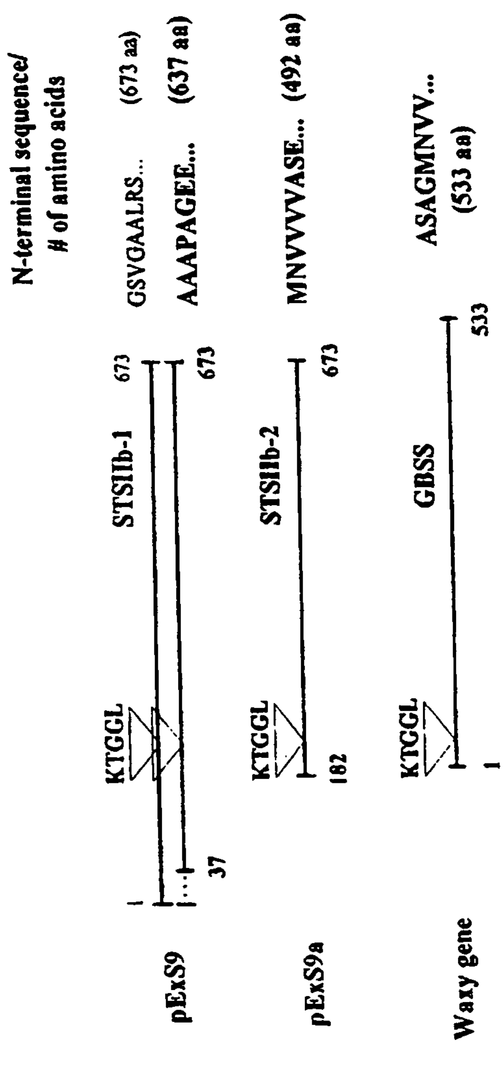

N-terminal sequence/
# of amino acids
pExS9
pExS9a
Waxy gene
KTGGL
KTGGL
KTGGL
STSIIb-1
STSIIb-2
GBSS
1
37
673
673
182
673
1
533
GSVGAALRS... (673 aa)
AAAPAGEE... (637 aa)
MNVVVVASE... (492 aa)
ASAGMNVV...
(533 aa)

Fig. 60

Fig. 61

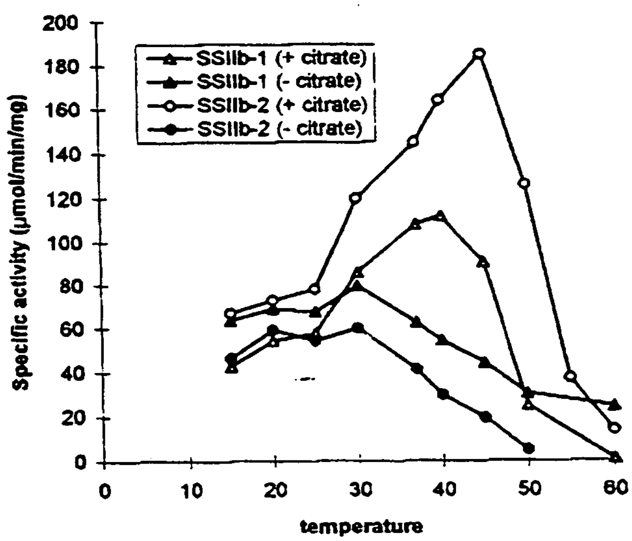

SSIIb-1 (+ citrate)
SSIIb-1 (- citrate)
SSIIb-2 (+ citrate)
SSIIb-2 (- citrate)
Specific activity (µmol/min/mg)
200
180
160
140
120
100
80
60
40
20
0
0
10
20
30
40
50
60
temperature

Fig. 62

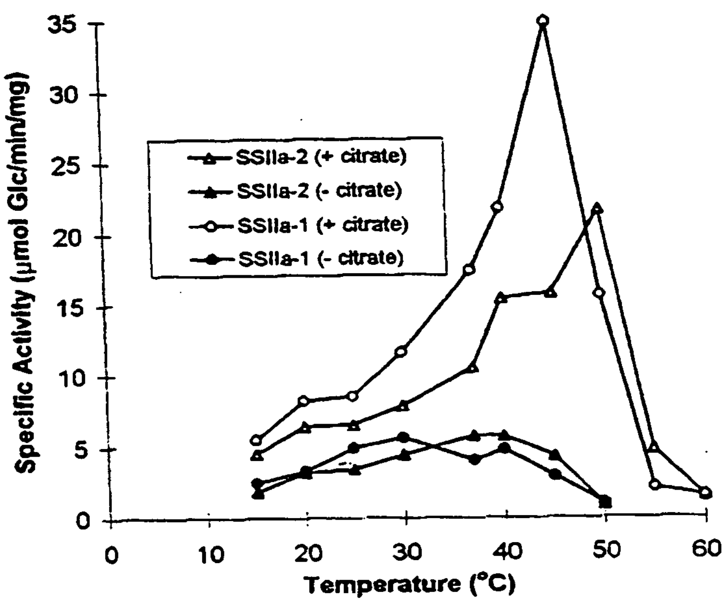
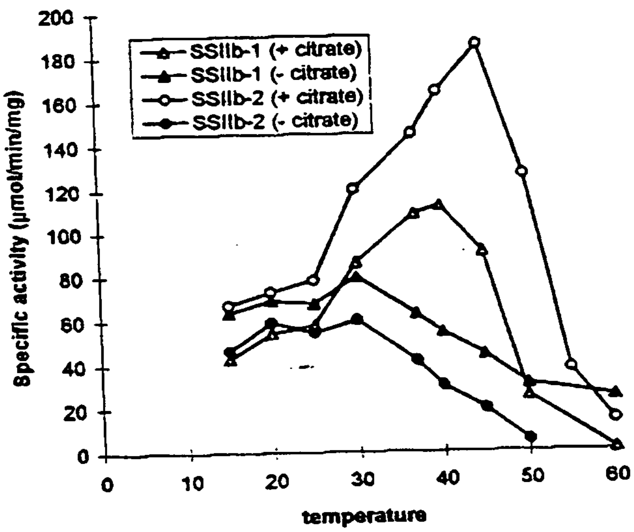

SSIIa-2 (+ citrate)
SSIIa-2 (- citrate)
SSIIa-1 (+ citrate)
SSIIa-1 (- citrate)
Specific Activity (µmol Glc/min/mg)
Temperature (°C)
0
5
10
15
20
25
30
35
0
10
20
30
40
50
60

Fig. 63


SSIIb-1 (+ citrate)
SSIIb-1 (- citrate)
SSIIb-2 (+ citrate)
SSIIb-2 (- citrate)
Specific activity (µmol/min/mg)
200
180
160
140
120
100
80
60
40
20
0
0
10
20
30
40
50
60
temperature

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US98/06660

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :Please See Extra Sheet.
US CL :Please See Extra Sheet.
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/69.1, 71.1, 101, 172.3, 194, 252.3, 252.33, 254.11, 254.2, 320.1, 419; 800/205, DIG 9, DIG 52, DIG 55

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X -- Y	OKITA et al. Biosynthesis of Bacterial Glycogen: Cloning of the Glycogen Biosynthetic Enzyme Structural Genes of Escherichia Coli. The Journal of Biological Chemistry. 10 July 1981, Vol. 256, No. 13, pages 6944-6952, see pages 6944 and 6946.	3,7,9,16,17,27,28 ---------------- 1,2,10-15,32-36
Y	US 5,348,675 A (ULLMANN et al) 20 September 1994, column 1, lines 9-59.	1-3,7,9-17,26-28,32-36
Y	US 5,608,149 A (BARRY et al) 04 March 1997, column 8, lines 5-36; column 14, lines 5-67; column 18, lines 60-67; column 21, lines 36-67; column 22; column 24; column 25, lines 29-33; column 26, lines 32-59; column 38, lines 20-25.	1,3-6,8,9,15

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search
01 JULY 1998

Date of mailing of the international search report
19 AUG 1998

Name and mailing address of the ISA/US
Commissioner of Patents and Trademarks
Box PCT
Washington, D.C. 20231
Facsimile No. (703) 305-3230

Authorized officer
DAVID T. FOX
Telephone No. (703) 308-0196

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 5,300,145 A (FERGASON et al) 05 April 1994, column 3, lines 3-68.	29-31,37,38
Y	WO 92/14827 A1 (INSTITUT FUR GENBIOLOGISCHE) 03 September 1992, pages 1-3, 9-10, 12, 15-21.	11-15,23-25,36,38
Y	IGLESIAS et al. Expression of the Potato Tuber ADP-glucose Pyrophosphorylase in *Escherichia coli*. The Journal of Biological Chemistry. 15 January 1993, Vol. 268, No. 2, pages 1081-1086, see page 1081.	1,3,7,15,16,27,28
Y	TANAKA et al. Structure, Organization, and Chromosomal Location of the Gene Encoding a Form of Rice Soluble Starch Synthase. Plant Physiology. June 1995, Vol. 108, No. 2, pages 677-783, see page 677.	3,10,18,20-22,26,35
Y	JAMES et al. Characterization of the Maize Gene *sugary1*, a Determinant of Starch Composition in Kernels. The Plant Cell. April 1995, Vol. 7, No. 4, pages 417-429, see pages 417-418.	3,10,18,19,36
Y	HOVENKAMP-HERMELINK et al. Isolation of an amylose-free starch mutant of the potato (*Solanum tuberosum* L.). Theoretical and Applied Genetics. 1987, Vol. 75, pages 217-221, see page 217.	18-25
Y	LIN et al. Isolation and Characterization of a Starchless Mutant of *Arabidopsis thaliana* (L.) Heynh Lacking ADPglucose Pyrophosphorlyase Activity. Plant Physiology. 1988, Vol. 86, pages 1131-1135, see page 1131.	18-25

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US98/06660

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
   because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
   because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
   because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1. ☒ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☒ No protest accompanied the payment of additional search fees.

A. CLASSIFICATION OF SUBJECT MATTER:
IPC (6): A01H 5/00; C12N 15/29, 15/31, 15/54, 15/70, 15/74, 15/80, 15/81, 15/82; C12P 19/04

A. CLASSIFICATION OF SUBJECT MATTER:
US CL : 435/69.1, 71.1, 101, 172.3, 194, 252.3, 252.33, 254.11, 254.2, 320.1, 419; 800/205, DIG 9, DIG 52, DIG 55

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING
This ISA found multiple inventions as follows:

This application contains the following inventions or groups of inventions which are not so linked as to form a single inventive concept under PCT Rule 13.1.

Group I, claim(s) 1-3, 7, 9-17, 26-28 and 32-36, drawn to bacterial or fungal hosts transformed with a plasmid comprising starch synthesis genes, and a method for their use.

Group II, claim(s) 1, 3-6, 8-17, 26 and 34-36, drawn to plants transformed with a plasmid comprising starch synthesis genes, and a method for their use.

Group III, claim(s) 18-25, drawn to a host which is deficient for the activity of two particular starch synthesis enzymes.
Group IV, claims 29-31 and 37-38, drawn to polysaccharides.

The inventions listed as Groups I-IV do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

The invention of Group I, drawn to a first product and method of use, shares the technical feature of requiring transformed microbes, microbial-functional gene regulatory sequences, and processes for microbial transformation; these features are not required by any other group.

The invention of Group II, drawn to a second product and method of use, shares the technical feature of requiring transformed plant cells and plants, plant-functional regulatory sequences, and processes for plant cell transformation and whole plant regeneration; these features are not required by any other group.

The invention of Group III, drawn to a third product, shares the technical feature of requiring mutant or gene- or enzyme- inactivated host cells, and methods for such mutation or gene- or enzyme- inactivation; these features are not required by any other group.

The invention of Group IV, drawn to a fourth product, shares the technical feature of being isolated polysaccharides, this feature is not required by any other group.